PERFORMANCE OF CYANOGENIC AND ACYANOGENIC PLANTS AND PROGENIES OF *Trifolium repens* L.

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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1976

ACKNOWLEDGEMENTS

The author is deeply indebted and thankful to Dr. C. E. Dean, Chairman of the Supervisory Committee for the technical assistance and guidance during the course of this dissertation. Appreciation is also extended to Dr. E. S. Horner, Dr. V. N. Schroder, Dr. D. H. Hubbell and Dr. D. E. Purcifull for their professional assistance as members of the Supervisory Committee.

The author wishes to express his appreciation to Dr. F. T. Boyd for assistance at beginning of this research, and to Dr. F. G. Martin for the statistical analysis.

The technical assistance in the field provided the author by Mr. W. H. Anderson and that of the staff and personnel of the Agronomy Farm and Forage Laboratory is sincerely acknowledged.

The financial assistance provided by the USAID/University of Wisconsin - Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, and by the Departamento de Fitotecnia - UFRGS is gratefully acknowledged.

Finally, the author wishes to thank his wife Maria Isabel and son, Felipe, for accepting with patience their neglect during the course of this study.

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Abstract of Dissertation Presented to the Graduate
Council of the University of Florida in Partial Fulfillment
of the Requirements for the Degree of Doctor of Philosophy

PERFORMANCE OF CYANOGENIC AND ACYANOGENIC PLANTS AND PROGENIES OF *Trifolium repens* L.

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August, 1976

Chairman: Dr. Charles E. Dean
Major Department: Agronomy

Natural populations and introduced breeding lines of white clover (*Trifolium repens* L.) were surveyed for the presence and absence of the cyanogenic glucosides and the associated hydrolytic enzyme (linamarase) during the spring of 1974 in Gainesville, Florida. The majority of the natural plants selected and analyzed were cyanogenic and the majority of the introduced breeding lines were acyanogenic.

Forty clones, replicated eight times as single plants and including the cyanogenic genotype (Li_Ac_), genotype with only the enzyme linamarase (Li_acac), genotype with only glucosides (liliAc_), and genotype without both the enzyme and glucosides (liliacac) were used in a polycross nursery in 1975. The following measurements were made: diameter and height of plants in cm, length of stolon internodes in cm, virus symptom rating according to the scale 0 to 4 from absence to very strong symptoms, flowering time in days after planting, seed yield in grams per plant, and life span of the plants in days after planting. With respect to the factors glucosides and enzyme, all the differences observed in the responses studied showed significant effects. In general, cyanogenic plants were larger in plant diameter, had shorter stature, longer stolon internodes, flowered earlier, and had shorter life span as compared with acyanogenic plants. A wide variation within genotypes was observed. Except for the virus symptom rating, the effects of the glucosides and the enzyme were found to interact.

A progeny test of 38 clones was conducted in 1976 at the Beef Research Unit, Gainesville, Fla. The following measurements were made: hydrocyanic acid (HCN) content in ppm, flowering time in days after planting, length of stolon internodes in cm and dry matter yield in kg/ha, separated into two components, white clover and grasses. With respect to the factors glucosides and enzyme, few of the differences observed with the responses measured were significant. The progenies of the cyanogenic genotype flowered earlier and had a higher HCN content than the other genotypes.

The clover dry matter yield for all progenies showed no significant differences.

The results obtained in this study indicated that the use of cyanogenic plants might be useful in development and adaptation of varieties to warm conditions. Since higher levels of HCN are associated with earlier flowering, this characteristic could be useful in some of the Ladino types which flower very little under southern conditions. The amount of hydrocyanic acid (HCN) was diluted in the progenies to a lower level after the intercrosses among cyanogenic and acyanogenic plants.

INTRODUCTION

The use of forage pastures for the grazing animal should have first priority in livestock production. Forage is a food, generally not suitable for direct human consumption but very suitable for the adapted ruminant animal, which should not compete with man for the same source of nourishment. In pasture and forage programs, legumes play a very important role by providing nutritious forage during the year and fixing significant amounts of atmospheric nitrogen, thus reducing the amount of N which must be applied in a fertilization program.

White clover (*Trifolium repens* L.) is one of the most widely adapted winter legumes. Many different forms are found growing indigenously or cultivated, throughout the world. They represent a large source of genetic variation which can be used in a breeding program for adaptation and development of new varieties. One of the genetic characteristics studied in white clover is hydrocyanic acid (HCN), which is evolved from some genotypes when their leaves are damaged. The amount of HCN released from white clover is very small, causing no toxic effects on large animals. It is thought, however, that it is related to the adaptation of varieties to warm conditions.

In this study an attempt was made to characterize different populations of white clover involving individual plants and progenies of different genotypes in relation to the presence or absence and content of HCN. Different responses were measured and the possible relation among genotypes studied with the objective of providing information which might be helpful in future breeding programs.

LITERATURE REVIEW

Species Characterization and Distribution

White clover (Trifolium repens L.) originated in the eastern Mediterranean countries or Asia Minor (Gibson and Hollowell, 1966). It has spread from there into Europe to approximately 71°N Lat. and to Asia by more or less natural means, and was introduced into America, Australia, and New Zealand by early settlers. White clover is now found in all temperate parts of the world where conditions are suitable (Davies, 1969).

The great range of morphology in white clover is recognized in the classification into agronomic types small, intermediate, and large. These are represented by the varieties Kent Wild White, Louisiana White, and Ladino, respectively. This classification was particularly valuable in describing early varieties and ecotypes. There are not many varieties of white clover of American and Canadian origin. Many of them are ecotypes and are not varieties developed from a specific plant breeding program. Two variety names used in writings and advertisements are misleading and erroneous. These are Dutch White Clover and Wild White Clover. The name Dutch was applied to any white clover, the seed of which was produced in the Low Countries. Dutch in common usage is a synonym for Common White, which may be any of the three "agronomic types" or mixtures thereof. The term "Wild White" apparently originated in Great Britain, and was used there to designate the small type.

Ladino, as recorded in 1848, was first found growing on an estate in the Po Valley of Italy. It is a variety of the large type of T. repens L. In general, all its vegetative parts are larger than those of intermediate white. Many varieties were developed from Ladino type, such as the Merit and Pilgrim synthetic varieties, and by crossing Ladino with other varieties, such as the Nordic variety (Gibson and Hollowell, 1966).

Louisiana S-1 is a synthetic variety of the intermediate type. It is higher yielding and more persistent during summer months than the parent strain Louisiana White and is particularly adapted to the Gulf Coast states (Hollowell, 1958).

Tillman white clover is a 6-clone synthetic variety from Ladino white clover of diverse origin selected for profuse branching stolons, sparse flowering, persistence of stands, general disease resistance, and improved forage production in South Carolina. Plants and seed of Tillman and Ladino are indistinguishable but Tillman is superior in persistence of stands and forage production (Gibson, Beinhart, and Halpin, 1969).

Regal white clover is a synthetic variety developed from Ladino types. Clones were selected in polycross performance trials using persistence, summer production, and forage yield as criteria. In appearance, Regal is not readily distinguishable from other Ladino varieties. The outstanding characteristic of Regal is higher forage yield as a result of more summer production and greater persistence (Johnson, Donnelly, and Gibson, 1970).

Cyanogenesis in white clover has attracted the attention of workers in both pure and applied research since HCN, which is produced as a result of the hydrolysis of cyanogenic glucosides by an enzyme, may have toxic effects in animals. Corkill (1940) classified white clover with regard to cyanophoric properties into four distinct groups: (a) plants containing the cyanogenetic glucosides and also the appropriate enzyme which hydrolizes these glucosides and produces hydrocyanic acid, (b) plants which contain the glucosides but not the enzyme, (c) plants which contain the enzyme but not the glucosides, and (d) plants which contain neither the glucosides nor the appropriate enzyme. The genes for cyanogenetic glucosides and the hydrolytic enzyme were investigated in wild white clover populations of Europe and the Near East, including Alpine populations and cultivated strains (Daday, 1953). The four chemical phenotypes were distinguished as follows: (1) glucosides and enzyme (G+E), (2) glucosides only (G), (3) enzyme only (E), and (4) neither glucosides nor enzyme (O). Considerable variation was found in the occurrence and proportion of the four phenotypes in populations from different parts of Europe and the Near East. A transformation in the phenotypic structure of populations became evident, and samples revealed a change from south to northeast. A continuous gradual decrease of the dominant cyanogenetic glucosides and enzyme gene frequencies was established within a range of 0 to 100%. A similar change in the phenotypic structure and of decreasing gene frequency was found in the Alpine population. The cultivated white clover showed phenotypic and genotypic structure similar to the wild populations from which they originated, but a certain divergence was revealed in genetical structure because of selection by plant breeders.

There was a continuous gradual decrease in frequencies of the genes for the glucoside lotaustralin and the enzyme (linamarase) over the whole range from 100 to 0% as the source of samples of wild T. repens L. populations moved from the Mediterranean region to northeastern

Europe. The distribution of the dominant allele frequencies in the populations was closely correlated with January isotherms. A decrease of 1°F in January mean temperature resulted in a reduction of 4.23% in the frequency of the glucoside gene, and a reduction of 3.16% in the frequency of the enzyme gene. Thus, January temperatures have played an important role through natural selection in the evolution of subspecies in _T. repens_ L. (Daday, 1954a and 1954c).

The genetic structure of _T. repens_ L. populations derived from different altitudes in the Central European Alps showed wide variation (Daday, 1954b). The dominant glucoside lotaustralin allele (Ac) frequency varied from 0.6241 at 1903 feet to nil at 6398 feet, and the dominant enzyme linamarase allele (Li) likewise varied from 0.5701 at 1903 feet to 0.0204 at 6398 feet. This decrease was shown to be correlated with the decreasing January temperature at high altitudes.

In Great Britain the wild white clover populations are characterized by high frequencies of dominant genes for the presence of glucosides and enzyme, in contrast to northern Netherlands, Denmark, Sweden and northern Italy, which have low dominant gene frequencies (Daday, 1955).

Cyanogenic Polymorphism

Trifolium repens L. is polymorphic for the cyanogenic character. Plants that evolve hydrocyanic acid (HCN) after mechanical damage to the leaves are called cyanogenic, and those without this property are acyanogenic. Mirande (1912) was the first to report the presence of cyanoglucoside in white clover, and subsequently various workers have shown that different strains of white clover vary in their glucoside content.

Considerable correlation between HCN content and type in white clovers found in New Zealand was shown, with the best and highest producing agronomic types being highest in HCN content (Doak, 1933). The New Zealand Department of Agriculture, in their program of white clover certification, used the HCN content as a means of identifying the various types (Foy and Hyde, 1937).

Cyanogenesis depends primarily on the presence of the two "cyanogenic glucosides" linamarin and lotaustralin. Two independent enzymes are known to be involved in the production of HCN. One enzyme mediates the production of the glucosides from some precursor, while the second enzyme (linamarase) brings about the breakdown of the glucosides (Corkill, 1952). The presence and absence of the enzyme linamarase in white clover is governed by the pair of genes Li, li; the presence of the enzyme is dominant. The presence and absence of the glucosides are governed by the pair of genes Ac, ac; the presence of the glucosides is dominant. The cyanogenic character of white clover is governed by the interaction of the genes for the presence of the enzyme linamarase and the glucosides. Cyanogenic plants contain Li and Ac, while acyanogenic plants may contain either one or neither of the dominant genes Li and Ac (Corkill, 1942).

The cyanogenic glucosides of white clover have been isolated and the relative proportion of linamarin to lotaustralin varies between 26%:74% and 50%:50%.

Intraplant (intragenotype) variability in the HCN content of clover leaves was demonstrated as a seasonal variation. The HCN content of leaves was also shown to vary both with increasing size of the plant and with a decrease in the water supply. The HCN content decreases with increasing individual leaf age. This variation in hydrocyanic acid release is reflected in a variation in linamarase activity of the leaves (Hughes, 1969).

The metabolism of cyanogenic glucosides may be considered in two parts: (1) synthesis of cyanogenic glucosides, and (2) cyanide metabolism. The role of amino acids as precursors of the cyanogenic glucosides has now been established, using ^{14}C labelled amino acids, in the following species: (a) Isoleucine to lotaustralin in *Trifolium repens* L. and *Linum usitatissimum* L., (b) Valine to linamarin in *T. repens* L. and *L. usitatissimum* L., (c) Tyrosine to dhurrin in *Sorghum vulgare* Pers., and (d) Phenylalanine to prunasin in *Prunus laurocerasus* L. With ^{14}C applied as $H^{14}CN$ to seedlings of *S. vulgare* Pers. an extensive and specific conversion of ^{14}C to the amide C atom of asparagine was demonstrated (Hughes, 1969). Similar results have been obtained with *L. usitatissimum* L., *T. repens* L. and the noncyanogenic species barley, pea, and wheat. The primary product of HCN assimilation is the compound B-cyano-L-alanine; the other three carbon atoms are supplied by cysteine and the reaction is catalysed by the enzyme B-cyano-L-alanine synthetase. In view of these findings, it is quite clear that the cyanogenic glucosides are metabolically active rather than being the inert end products of metabolism. It is now obvious that these glucosides are intimately connected with some aspects of amino acid metabolism, although the exact physiological significance of this to the plant remains an open question.

Cyanogenesis in different species has been studied by several workers. Association of HCN potential with many plant characteristics has been reported for *Sorghum bicolor* (L.) Moench. and *S. sudanense* (Piper) Stafp (Nass, 1972). In the literature, dominance or partial dominance for genetic control of both high and low HCN potential is indicated in these crops. The number of genes regulating HCN production in sorghum and sudan-grass has not been established.

Cyanogenesis in *Lotus corniculatus* L. was found to be determined by a dominant gene which gives tetrasomic inheritance in the F_2. Plants used in this genetic investigation segregated sharply into cyanogenic and acyanogenic classes. Some other plants were found to give off only traces of hydrogen cyanide. This is presumably due to the action of modifying factors. *L. corniculatus* L. and *L. tenuis* Wald & Kit. have cyanogenic and acyanogenic forms in wild populations. *L. uliginosus* Schkuhr., *L. angustissimus* L., and *L. hispidus* Desf. have never been found to be cyanogenic (Dawson, 1941).

Trifolium repens L. and 16 closely related species were tested qualitatively for the presence of glucosides and for the enzyme that releases HCN by hydrolysis of glucosides in damaged leaves. In contrast to *T. repens* L., none of these species contained the enzyme in the absence of glucosides (Gibson, Barnett, and Gillingham, 1972).

A selective preference for acyanogenic plants by herbivores has been observed. Leaves of acyanogenic and cyanogenic *Lotus corniculatus* L. and *Trifolium repens* L. were presented to 13 species of slugs and snails. An estimate of the quantity of leaves consumed in each feeding trial was obtained from the dry weight of the plant faeces produced. Evidence supports the hypothesis that molluscs selectively eat acyanogenic plants and, therefore, that the cyanogenic character acts as a defensive mechanism against herbivores. Species of mollusc differ greatly in the degree to which they exhibit differential eating preference and, in general, the phenomenon appears to be more a feature of *L. corniculatus* L. than of *T. repens* L. It is suggested that this form of selection is more likely to operate upon seedlings than upon established plants (Crawford-Sidebotham, 1972).

In another study (Whitman, 1973), quantitative measures of feeding damage by herbivores and cyanogenesis were obtained from 1000 *T. repens* L.

plants growing in unmanaged areas. An association between low herbivore feeding damage rating and cyanogenesis was indicated. If only those plants given the highest feeding damage rating have significant reduction of vegetative growth and/or seed production, this report would help to explain the maintenance of cyanogenesis polymorphism in *T. repens* L.

Angseesing and Angseesing (1973) reported a selective preference for acyanogenic plants by herbivores. Angseesing (1974) confirmed these results with choice chamber experiments which showed that the counter-balancing selection against acyanogenic plants is due to selective preference by at least two herbivores (*Arion ater* L. and *Agriolimax reticulatus* Muller). However Miller et al. (1975), in seedling tests in different locations, in the field or with flats exposed to the field conditions, without choice control, could find no selectivity by herbivores. They concluded that some force other than selective elimination of seedlings accounts for the high frequency of cyanogenic white clover plants in warm climates.

Hydrocyanic Acid Determination

A qualitative technique for determination of HCN in *Phaseolus lunatus* L. was developed by Guignard (1906), using filter paper soaked in a 1% solution of picric acid and a 10% sodium carbonate solution. Basically the Guignard picrate-paper test consists of comparing standards prepared by using different concentrations of HCN, and consequently with different colors, with the results of the samples in study. This method, with modifications, has been widely used since that time.

Boyd et al. (1938) described a method that involves heating of an alkaline picrate solution with distillate from Sudan grass and a visual comparison of the color change with standards. Doak (1938) in a private communication received more details from F. T. Boyd of the Everglades Experiment Station, Florida, and in a footnote to a paper, endorsed the Boyd procedure for white clover. Later, Jackson (1958) recognized the importance and efficacy of the Boyd procedure.

Sullivan (1939) described an adaptation of the Boyd method to the determination of hydrocyanic acid in individual plants of white clover. Of all the plants studied 70% contained 0.001 mg or less of hydrocyanic acid on the fresh weight basis. Owing to the limited quantity of material available, a determination of less than 0.05 mg of hydrocyanic acid was often necessary. He suggested that such small quantities could be more accurately determined by the KWSZ photometer than by visual comparison with standards.

The glucosides were isolated by Melville and Doak (1940) and found to be a mixture of linamarin and lotaustralin, and were shown by Coop (1940) to be hydrolyzed by linamarase prepared either from linseed or from the clover plant; Sullivan (1944) proposed a modification of the picric acid method for determination of hydrocyanic acid in white clover plants. He recommended disintegration of the leaves by grinding, addition of a quantity of linamarase and toluene, and incubation in a closed flask for at least one day before distillation.

Gilchrist, Lueschen, and Hittle (1967) proposed a method for the preparation of standards in the sodium picrate assay of HCN. The method relies on recovery rather than solution standards. The recovery standards are prepared by releasing HCN from stock cyanide solutions, recovering the HCN on alkaline picrate paper strips, followed by elution in distilled water.

Burns et al. (1970) presented a procedure for the quantification of potential HCN released from green tissue. The procedure was suggested in preference to qualitative procedures because resulting data can be compared among both laboratories and regions. In addition, results from qualitative procedures frequently appear incorrectly on a quantitative basis. The procedure was evaluated for its precision and recovery. An experiment was conducted to examine method(s) of sample preservation and period(s) of sample holding and incubation prior to analysis. Forage sealed in a polyethylene bag and immediately frozen gave HCN contents similar to samples stored in the dark at 26°C. Once preserved, samples were held for 6 hours prior to analysis without significant reduction in HCN levels; however, a slight reduction was noted after 3 hours. Maximum release of HCN was obtained after samples had been incubated in ethanol for 24 hours with no significant change through 96 hours.

The picrate test is very useful in identification of genotypes for the presence or absence of the glucosides and/or enzyme. The test in white clover is made on the first but folded leaf, to which two drops of toluene are added together with two drops of either the glucoside or enzyme extract. A simple water extract of glucoside is made from a plant genotype liliAcAc, using a pestle and mortar. Similarly the enzyme is extracted in citrate phosphate buffer at pH 5.0 from a LiLiacac plant. The tubes are incubated at 35°C for 12 hours (Hughes, 1969).

Some workers have studied the activity of the linamarase enzyme (Hughes and Maher, 1973). Evidence has been sought on the possible existence of multiple forms of the enzyme controlled by the Li locus in white clover. During purification of enzyme from LiLi plants, there was no separation of activities against the B-glucosides, p-nitrophenyl B-D-glucoside, salicin, and linamarin-lotaustralin, and the B-galactoside, p-nitrophenyl B-D-galactoside. In addition, tests on mixtures of these four substrates provided no evidence for the existence of more than one enzyme. Immunological tests have shown that plants homozygous for the recessive li allele do not contain an enzymatically inactive protein, antigenically related to the normal enzyme. This suggests that li alleles either specify a low-activity, immunologically altered protein or control the synthesis of very low levels of normal enzyme.

Maher and Hughes (1973) tested various reagents which prevent enzyme inhibition by phenolic compounds in attempts to improve the medium used to extract the Li-controlled enzyme activities from white clover leaves. The addition of 50 mM diethyldithiocarbamate to the extraction medium gave a five fold increase in the enzyme activity of LiLi white clover extracts against p-nitrophenyl B-D-glucoside, linamarin-lotaustralin, and p-nitrophenyl B-D-galactoside. These three substrates were used in tests on the effect of genotype on enzyme activity. An absence of dominance at the Li locus was demonstrated with a dosage effect of Li alleles on enzyme activity. A new Li allele was identified in the Lili clone, C11, which had low levels of enzyme activity. In crosses with two lili clones, Li(C11)li progeny were produced with activity levels similar to those of the C11 parent. Inhibition and heat-inactivation tests suggested that the lili clone, D4, produces an altered form of B-glucosidase which may also be present in LiLi plants. Extracts of the two homozygous LiLi clones, C2 and C5, had considerably higher B-glucosidase activity than extracts of nine Lili clones. This suggests the possibility of an absence of dominance at the Li locus, with a double dose of Li allele causing higher specific activity levels than a single dose.

White Clover Breeding

Breeding techniques must take into consideration the biology of the plant and the mode of its usage. Two salient points in relation to white clover are that it needs to be cross-pollinated by insects and that there is a wide range of management techniques of grass/clover swards.

Attention has been drawn frequently to the highly polymorphic and heterogenous nature of the species and the great variation that exists in natural populations (Davies, 1969).

Characteristics of white clover that affect the choice of techniques and methods to be used in developing improved varieties are listed by Gibson and Hollowell (1966). Plants are highly self-incompatible, enforced by a series of S alleles, but selfing is possible by using either the Sf gene or the pseudoself-compatibility characteristic. Plants are highly heterozygous as a result of this enforced cross-polination. Plants normally bloom and set seed every year, and flowering for making crosses may be induced easily in the greenhouse. Hand pollination is easy and results in a good seed set. Vegetative propagation to the extent needed in a breeding program is easily done by rooting stolons. Plants of clones may be grown and maintained in the greenhouse in 6-inch pots or smaller containers. These workers also listed the three qualitative characters that have been studied in considerable detail, namely leaf markings, incompatibility and cyanogenetic properties.

Graumann (1952) considered that the relative value of diallel crosses to determine both specific and general combining ability and of inbreeding to study genetic stability are well recognized. However, these procedures, especially the former, impose definite restrictions on the number of plants which may be appraised when working with species characterized by floral habits which are unadapted for large-scale controlled crossing by the bagging method, as in corn. It is with many forage species which are in this category that the polycross method offers the greatest advantage. This method provides opportunity, at reasonable costs and with limited land area, to produce the seed needed to get a reliable measure of general combining ability of a large number of individuals with sufficient offspring from each to permit comprehensive trials.

Gibson et al. (1963) evaluated two morphologically contrasting types of white clover plants designated as viny and nonviny, for persistence and forage production by growing field plots established with clonal propagules and polycross progenies. The nonviny type was superior in both persistence and forage production. Selection for the nonviny type apparently is justified. Evaluation of clones on the basis of performance of clonal propagules generally agreed with evaluation on the basis of performance of polycross progenies.

Beinhart et al. (1963) observed stolons of viny and nonviny white clover clones in a study of morphological differences affecting leaf area production. The most important differences were that stolons of nonviny plants contributed to greatly increased leaf area and, presumably, to increased growth rates. Individual stolons had short life spans. Branches frequently survived the death of parent stolons and remained as vegetative propagules. Because continual production of new vegetative meristems is necessary for stand survival, the branching frequency of a clone may be a useful predictor of its persistence potential. Increased summer branching thus may lead to both greater growth and improved persistence of white clover.

Gibson (1964) developed a technique requiring few seeds for evaluating white clover strains. He compared check-planting white clover by transplanting individual seedlings at spacings of 6 and 12 inches with broadcasting seed at the rate of 3 pounds per acre as spacings for establishing field plots. He concluded that check-planting white clover at the 6-inch spacing provides a valid evaluation of strains and varieties. Since this method requires relatively few seed, producing the seed by making hand pollination in a greenhouse is feasible.

Hovin (1963) studied the compatibility reactions of interspecific hybrids between _T. repens_ L. and _T. nigrescens_ Viv. His results indicated that self-fertility had been transmitted from the self-fertile _T. repens_ L. parent. The self-compatible hexaploid and one self-incompatible hexaploid were cross-compatible with a self-incompatible plant used as seed parent. Segregation in F1 and F2 suggested that independent action of S alleles and possibly dominance in heterogenic pollen conferred cross-fertility.

Cohen and Leffel (1964) evaluated white clover clones for pseudo-self-compatibility by manipulation of flowering heads. Significant differences for pseudo-self-compatibility were found repeatedly among clones, which tended to exhibit relative differences in pseudo-self-compatibility regardless of date of determination. Manifestation of pseudo-self-compatibility within S1 progenies appears to be independent of specific S locus genotypes.

The production of polyploids has been used as a method of breeding as well as a technique for studies of genetics and incompatibility in autotetraploid white clover (Brewbaker, 1954 and 1955; Evans, 1955). However, white clover tetraploids have not yet been successful, while the tetraploids in other species have given a higher yield in green and dry weight than diploids (Turesson, 1962).

Adaptation and Production

White clover is one of the most nutritious and widely distributed forage legumes of the world. It is a member of the flora of every continent, where it is widely distributed. In the Western Hemisphere, its distribution extends from Alaska to southern South America (Gibson and Hollowell, 1966). Ecotypes of Mediterranean origin in higher latitudes generally show higher productivity during late autumn and early winter than the indigenous or developed varieties in those regions. Such introductions usually show rather poor winter hardiness. They are, however, valuable as a source of genetic variation for other characters which could be used to improve existing adapted varieties (Connolly, 1969).

In north Florida, white clover varieties vary in their dependability and ability to produce forage. Louisiana S-1 and Nolin's Improved are two of the most dependable for reseeding. Ladino types produce high yields but do not flower and seed satisfactorily for reseeding because of day length (Killinger, 1970). According to Kretschmer (1966), there does not appear to be at present another self-regenerating clover for use in improving white clover in Florida pastures.

Environmental factors are very important in adaptation and production of white clover. Blaser and Killinger (1950) studied the germination of volunteer Louisiana white clover seed in natural soil environments, as influenced by different temperatures, length of exposure, alternating temperatures, and sod management. Germination was very poor at a continuous temperature of 71°F and variable at higher temperatures. Six hours of exposure to 37°F and 3 hours to 0°F, with subsequent higher temperatures, increased germination. Germination was increased as the time of exposure at 37°F was increased to 24 hours. Exposure of the soil samples with volunteer seed to four cycles of alternating low-high temperature gave significant additional seed germination for each temperature cycle. Alternating temperatures improved seed germination, irrespective of the initial low temperature or duration of exposure.

Germination of volunteer and commercial Louisiana white clover seeds was compared under different temperature, moisture, and scarification treatments. Seed scarification eliminated the germination responses from low temperatures, and temperature changes were found to be effective only with moist environments. Close clipping of a grass sod encouraged early germination of white clover seeds. Mowing with subsequent burning-over was more effective in increasing the earliness of germination than mowing alone. The germination data suggest that in the absence of a perennial white clover population, the earliness of herbage production from seedling plants can be augmented by close grazing management during the fall.

Smith and Gibson (1960) studied the influence of temperature on growth and nodulation of white clover infected with bean yellow mosaic virus. The maximum growth and most conspicuous virus symptoms of white clover grown at 4 temperatures were obtained at 62°F. At 50°F the virus had no visible influence on growth. Root growth was greatest at 50°F. Inoculation with Rhizobium increased clover yields at temperature from 50° - 86°F, while virus infection decreased the beneficial effects of rhizobia at 62°F and above.

In another study Chen and Gibson (1973) observed the effect of temperature on pollen-tube growth in *T. repens* L. after cross and self-pollinations. They concluded that at low and intermediate temperatures (15° and 25°C), self-incompatiability in white clover is caused by abnormal growth of the pollen tubes. High temperatures enhance pollen tube growth and thus tend to break down or circumvent the incompatibility mechanism.

Soil moisture is another environmental factor very important in adaptation of white clover. Foulds and Grime (1972a) studied the influence of soil moisture on frequency of cyanogenic plants in populations of *T. repens* L. and *Lotus corniculatus* L. Their experimental work indicated that *T. repens* L. plants containing the cyanogenic glucosides should be infrequent in dry habitats. The results showed that the prediction is upheld for wild populations of *T. repens* L., but the polymorphism of cyanogenesis in *L. corniculatus* L. is not affected by drought conditions in the same way.

In another study Foulds and Grime (1972b) observed the response of cyanogenic and acyanogenic phenotypes of *T. repens* L. to moisture supply. Under experimental conditions of severe drought, fatalities in Ac phenotypes of *T. repens* L. exceeded those in ac phenotypes by a factor of three. Under sublethal moisture stresses the yields of Ac and ac phenotypes were not significantly different. Under moist conditions, ac phenotypes attained higher yields than the Ac plants suggesting a possible linkage between the Ac gene and genes affecting vegetative vigor. Cyanogenic plants displayed a low reproductive rate in all treatments. The effect of moisture stress was to inhibit flowering completely under conditions in which flowers were produced in all three acyanogenic phenotypes.

The effect of stage of growth at defoliation on white clover in mixed swards was studied by Margaret, Brockman, and Shaw (1970). They described four experiments in which various defoliation methods and frequencies were imposed on grass-white clover swards. Clover was not reduced by increasing the length of growth period or by cutting at a late stage of growth, but intensive grazing by sheep reduced clover compared with cutting, and taking a silage cut in a grazing sequence improved clover yield. These results do not support the contention that cutting a mixed sward for hay or silage supresses clover because of competition for light. With a "big white" type of clover infrequent defoliation may cause no more shading than frequent defoliation. Alternately, any additional shading may be compensated by the longer growth period. It is suggested that, in practice, competition for nutrients and moisture also contributes towards clover suppression when swards are cut for hay or silage.

Taylor et al. (1972) studied the establishment of white clover and alfalfa (Medicago sativa L.) from seeds sown on the soil surface of undisturbed Kentucky bluegrass (Poa pratensis L.) sod in later winter or early spring prior to the end of soil freezing and thawing. His data indicated that with doubling of the seeding rate, establishment of these legume species is likely to be as successful from surface seeding in undisturbed sod as with seed coverage and normal rates of seeding.

Flowering in white clover is variable and sometimes absent according to location and type of plants. Britten (1960) studied the genetic and environmental control of flowering in white clover in the tropics. White clover at low elevations expressed wide genetic variation in tendency to flower. Clones classified as flowering and nonflowering were subjected to temperatures associated with high elevations. Flowering in "nonflowering" clones was induced under warm-day cold-night treatments. It was proposed that in the tropics, low temperatures associated with high elevation are an important factor in determining flowering and therefore ability to persist in plants which are long-day and temperature sensitive.

Gibson (1957) studied the persistence of nonflowering, medium-flowering, and profuse flowering white clover under normal daylight and under an extended light period. Persistence was measured in terms of the number of live stolons transecting a line one meter long. The extended light period caused plants of the flowering group to reach a peak intensity of flowering earlier than usual and caused plants of nonflowering white clover to blossom profusely. The nonflowering clover under normal daylight period persisted longer than the same clover under the extended light period or the profuse flowering clover under the normal daylight period. The profuse-flowering clover under the extended light period persisted longer than the nonflowering clover under the same light period. Thus profuse-flowering clover under the extended light period had apparently passed the peak intensity of flowering and had reverted to vegetative growth prior to the occurrence of adverse summer conditions. In general, the reaction of the medium-flowering clover to an extended light period was intermediate to that of the nonflowering clover and the profuse flowering clover.

Bula et al. (1964) observed differences in vegetative and floral characteristics among white clover Syn. 1 generation progenies produced at diverse geographic locations. These differences are interpreted to reflect the differential growth responses of the parental clover at the respective locations. Progenies produced at the Prosser, Washington location appeared to reflect random pollination among six clones to a greater degree than progenies produced at the other locations. The differences among locations were not great enough to conclude unquestionably that progenies produced outside the region of origin differed from those produced within the region of origin. This conclusion may be restricted to the specific clones involved in these studies.

The soil mineral content has an effect on persistence and production of white clover. Woodhouse (1970) studied the effect of placement of phosphorous and lime on the growth of a ladino-orchardgrass sward. He stated that high exchangeable aluminum in subsoils has been suspected as a cause of limited persistence of ladino clover on some soils of the Southeast (U.S.). The effect on a ladino-orchardgrass sward of rather thorough incorporation of P and lime-stone into such a soil was studied. Mixing was by means of a rototiller, one furrow at a time, and extended as deep as 45 cm followed by disking to 15 cm. A conventional broadcast application was used for comparison. Two levels of each material were used, the level suggested by the soil test and 3 times this level. Deep incorporation resulted in no detectable benefit with either material, and appeared to reduce residual P. Subsoil Al was sharply reduced by liming and it appeared that mixing to a depth of 15 cm was adequate for these plants on this soil.

Whitehead (1970) determined carbon, nitrogen, phosphorous and sulphur in herbage, plant roots of white and red clover, lucerne, perennial ryegrass, cocksfoot, and timothy. His results indicated that decomposition of the root material would result in the mineralization of N in substantial amounts from white clover, in smaller amounts from red clover and in negligible amounts from lucerne. The roots of grasses would tend to immobilize soil N, even when they had received moderate applications of fertilizer N. The C:P ratio suggested that the legume roots neither mineralize nor immobilize inorganic phosphate but that the grass roots would induce immobilization. S was likely to be mineralized in appreciable amounts from roots of white and red clover, but not from roots of lucerne or the grass.

Margaret and Brockman (1970) investigated the effect of a range of N rates on herbage production from grass/white clover and grass swards in two long-term grazing experiments. The mixed sward yielded more than the grass swards over the range of fertilizer rates tested which were 0-120 lb of N/ac., but the response of the mixed sward to fertilizer N was less than that of the grass sward. This was due to the direct and indirect effect of clover, which was considerable with no fertilizer N, but decreased with increasing rates of application. However, clover was not completely suppressed by N at either site and contributed to herbage yields at all N rates.

Rate and time of N application and the amount of clover in the sward affected the seasonal distribution of dry matter yields. The mid-season decline was less marked at high levels than at intermediate levels of N supply.

MATERIALS AND METHODS

White Clover Selection and Clonal Propagation

Hydrocyanic Acid Analysis

The first concern of this study was to obtain plants having both high and low levels of HCN. Since preliminary analysis indicated that most varieties of white clover were low in HCN, it was decided to sample naturally occurring wild populations in an effort to obtain the necessary elevated levels.

It was of interest also to survey breeding lines, since much of this material could be expected to supply plants with low HCN levels. In addition, the level of HCN would also further characterize the lines.

The HCN determination was conducted on individual plants by the procedure described by Boyd et al. (1938) with some modifications. The method used is described as follows:

a) A weighed sample (10g) of fresh material (leaflets only) was placed in a distillation flask, to which was added 300 ml of distilled water and 5 ml of chloroform.

b) The distillate containing HCN was collected in a large test tube containing 10 ml of 1% KOH until a total of approximately 60 ml was obtained.

c) The volume of the distillate plus KOH was brought up to 100 ml in a volumetric flask by adding distilled water.

d) A 5 ml aliquot was taken and 5 ml of alkaline picrate solution was added to the aliquot. The mixture was heated in boiling water for 5 minutes to develop color, varying from yellow to dark brown, depending on the presence and amount of HCN.

e) The sample was cooled to room temperature, transferred to a colorimeter tube, and the percent transmittance on the colorimeter was read at wave length of 575 mμ.

f) The amount of HCN in ppm was estimated by comparison of the results with a standard curve (Appendix A-2) obtained with the procedure described by Sullivan (1939).

Glucosides and Enzyme Assays

Twenty clones from the samples that showed high HCN content and 20 with low HCN content were maintained in a greenhouse for further identification of the genotypes for the presence or absence of the glucosides and/or enzyme. The picrate test was employed for this purpose, using strips of Whatman No. 1 filter paper, 4.0 x 0.8 cm soaked in sodium picrate solution and suspended (wet) from the cork over two drops of chloroform in tubes approximately 6 cm long and 1 cm in diameter. Each plant sample consisted of an expanded but folded leaf, to which was added two drops of either the glucosides or enzyme extract. A simple water extract of glucosides was made from a plant with the genotype liliAcAc using a pestle and mortar. The enzyme was extracted in citrate phosphate buffer at a pH of 5.0 from a LiLiacac plant, using a mixture of 10.30 cm^3 of 0.2 M-Na_2HPO_4 and 9.70 cm^3 of 0.1 M-Citric Acid. The tubes were incubated at 35°C for 12 hours (Jones, 1966; Hughes, 1969). For each sample two test tubes were used, one with the enzyme extract and chloroform and the other with the glucosides extract and the chloroform. The samples with only the enzyme will change the color of the filter paper soaked in sodium picrate solution from yellow to brown, in the test tube that received the glucosides extract. The samples with only glucosides will change the color in the test tube that received the enzyme extract. The cyanogenic plants will change the color in the test tubes that received the enzyme and glucosides extracts. The genotype liliacac will not change the color in either one of the test tubes that received the enzyme and glucosides extracts. Using this procedure, five clones were identified with the genotype liliacac (O), five with the genotype Li_acac (E), seven with the genotype liliAc_ (G) and 23 of the genetic constitution Li_Ac_ (G+E). The forty clones were maintained in the greenhouse for future study.

Peanut Stunt Virus Identification

Virus infection was observed in the material in the greenhouse and was identified as a peanut stunt virus by serological tests with PVAS 62, antiserum from the American Type Culture Collection and normal serum from the Plant Pathology Laboratory, University of Florida.

Peanut stunt virus was described by Mink (1972) as a virus which causes pronounced stunting of peanuts and malformation of the fruit. The virus also causes epinasty, leaf distortion and stunt of beans, chlorotic mottle and stunt of Burley tobacco, and systemic mottle of several clovers, including *Trifolium repens* L. Physical, chemical, and serological properties along with kinds of transmission and other characteristics of peanut stunt virus are also reported.

Polycross Nursery

The polycross nursery was established on the Agronomy Farm, Gainesville, Florida, in a soil with pH 6.2 to which was added 400 kg/ha of 0-14-14 fertilizer and three units of Fritted Trace Elements (FTE). The forty white clover clones were planted 90 cm apart on December 18, 1974, in a randomized complete-block design with eight replications.

The following measurements were made on the experimental material: diameter of the plants in cm, height of the plants in cm, length of stolon internode in cm, and virus symptoms according to the scale 0 to 4 from absence to very strong symptoms. The flowering time was recorded to the date of the first open flower and computed as days after planting, and the life span of the plants was computed in the same way. Observations on flowering time and life span were made at least three times per week; seeds were harvested throughout the growing season and weighed separately for each plant.

Supplemental light was used to bring about more uniform flowering starting February 28, 1975 until the end of the experiment on July 7, 1975 from 7 to 11 p.m. with an average of 7.8 microeinsteins/m^2/second. On July 7, 1975 all plants were dead.

The data were analysed using an analysis of variance, and the differences among clones were partitioned into variation among genotypes and variation between treatments (clones) within genotypes. The two components were further subdivided. The first was partitioned to determine the effect of glucosides, enzyme, and their interaction on the seven responses, while the second was divided into the variation among treatments within each genotype to determine if differences existed between the treatments of a particular genotype.

The t-test was performed for the genotype means of responses that showed significant interaction with respect to the factors enzyme and glucosides. To further determine the nature of the treatment differences Duncan's multiple range test was performed on the treatment means within each genotype.

Some plants failed to flower, and the statistical analysis for this response was done in two ways: considering a zero value for these missing values and omitting the zero values.

Estimates of linear correlation were obtained among six of the seven responses. Virus symptom rating was omitted since this was a discrete variable, hence any correlation with this variable would have no meaning. Correlations were estimated separately for each genotype.

Progeny Test

Thirty-eight of the 40 clones of the polycross nursery produced enough seed for a comprehensive progeny test. They were distributed in the following genotypes: 5 liliacac (O), 5 Li_acac (E), 6 liliAc_ (G), and 22 Li_Ac_ (G+E).

The area used in this test was a typical flatwoods soil with a pH of 5.6, located on the Beef Research Unit, Gainesville, Florida. The area was covered with grasses belonging mainly to the *Paspalum*, *Axonopus*, and *Andropogon* genera. Two t/ha of dolomite lime was applied on October 21, 1975, and an application of 1000 kg/ha of 0-14-14 fertilizer with one unit of FTE was made on November 6, 1975. After the lime and fertilizer application on the surface of the mowed grass, a superficial scarification of the soil was done using a disk harrow.

Seed were planted in plots 1.8 m x 1.8 m, replicated 4 times in a randomized complete-block design. The seeds were sown on the soil surface at the rate of 5 kg/ha after inoculation with commercial *Rhizobium*, specific for white clover.

The area was irrigated until germination and establishment of the seedlings, and another application of 400 kg/ha of 0-10-20 fertilizer was made on April 23, 1976.

The following measurements were made on the experimental material: HCN content, using a 10 g fresh weight sample, by the method already described; flowering time was recorded to the date of the first open flower and computed as days after planting; length of stolon internode in cm; and dry matter yield in kg/ha. For the estimation of dry matter yield, an area of 1.35 m^2 in each plot was harvested, the green material was weighed, and a sample was taken for determination of botanical composition and dry matter. The sample material was separated into two components, clover and grasses, and was dried at 65°C for 48 hours. Three harvests were accomplished; the dates and progenies harvested each time are shown in table 1.

The very low temperatures which occurred in December of 1975 caused damage in the stands of many plots while they were in the seedling stage, and, because of this unusual situation, only the best progenies representative of each genotype were harvested each time.

The data were analysed using an analysis of variance, and the differences among progenies (treatments) were partitioned into variation among progenies of the different genotypes and variation between progenies within genotypes. The two components were further subdivided. The first was partitioned to determine the effect of glucosides, enzyme, and their interaction on the responses, while the second was divided into variation among treatments (progenies) from each genotype to determine if differences existed between the progenies from a particular genotype.

The t-test was performed for the progeny means of responses that

Table 1. Dates and progenies harvested during the experimental period, 1976.

Genotypes	Dates		
	April, 13	May, 11	June, 8
G+E	74-772 74-773 74-774 74-777 74-779 74-782 74-783 74-787	74-772 74-773 74-774 74-777 74-779 74-782 74-783 74-787 74-769 74-781	74-772 74-773 74-774 74-777 74-779 74-782 74-783 74-787 74-769 74-781
E	720c-8 516c-4	720c-8 516c-4 628-4	720c-8 516c-4 628-4
G	720c-5 74-780	720c-5 74-780 516c-7	720c-5 74-780 516c-7
O	74-559-1 715-9 719c-5	74-559-1 715-9 719c-5 716-7	74-559-1 715-9 719c-5 716-7

showed significant interaction with respect to the factors enzyme and glucosides. To further determine the nature of the treatment differences, Duncan's multiple range test was performed on the progeny means within each genotype.

RESULTS AND DISCUSSION

White Clover Selection and Clonal Propagation

Fifty-one samples, taken from natural populations at different locations in the Gainesville area during the spring of 1974, revealed seventeen very high in HCN. From 45 clones and/or progenies from varieties and introductions, six were found with high HCN content. The majority of these introductions are of the Ladino type. Daday (1955) also found very low frequency of cyanogenic plants in the Ladino type of white clover. These results agree with the evidence of high frequency of dominant genes in warm conditions.

The amount of HCN in all the samples analysed from natural populations and from the introduced material varied from 0.25 to 15.25 ppm. The percent transmittance data of the material maintained in the greenhouse are shown in Appendix 1. The material considered high in HCN ranged from 15.25 to approximately 4.25 ppm. Material considered low in HCN ranged from 4.00 to 0.25 ppm. The standard curve used is shown in Appendix 2.

The results of the picrate test for identification of genotypes revealed that the 20 clones considered high in HCN had the Li_Ac_ (G+E) genotype (cyanogenic phenotype). The 20 clones considered low in HCN were composed of the following genotypes: 3 Li_Ac_ (G+E), 5 liliacac (O), 5 Li_acac (E), and 7 liliAc_ (G). The last three groups showed acyanogenic phenotypes. The code clone designation, HCN content in ppm, and results of the picrate test are shown in Table 2.

The three clones that revealed presence of glucosides and enzyme from the material considered low in HCN showed a higher amount of HCN than the others in this group. One exception was found with clone 74-780, which had a HCN content of 1.75 ppm and presence of the glucosides only. Some variation may be expected because of the method used, and sometimes the enzyme may be present, but with low activity to break down the glucosides. Consequently, HCN is not detected by the picrate test, as was pointed out by Hughes, (1969). The possibility of absence of dominance at the Li locus also exists, and consequently a single dose of Li having a lower specific activity level than double dose as was described by Maher and Hughes, (1973).

The results of the serological test with ATCC PVAS 62 antiserum revealed that 13 clones out of 23 with Li_Ac_genotype were infected by peanut stunt virus, and 4 out of 17 liliacac, Li_acac, and liliAc_ genotypes were infected with the same virus. These results are shown in Table 3. The virus was found to be more widespread in the material selected from natural populations in the Gainesville area, where the virus presence was identified by Batchelor, Young, and Purcifull (1974).

Polycross Nursery

The results of the measurements made on the clones with respect to the responses studied, along with the analysis of variance, are shown in the Appendices A-3 to A-10.

With respect to the factors glucosides and enzyme, all responses showed significant effects. Except for the virus symptom rating, the effects of these two factors were found to interact. The nature of these interactions is shown in Table 4. Included are the genotype means along with the results of the t-test performed on each

Table 2. Clone designation, HCN content in ppm, and results of picrate test for phenotype identification.

Clones high in HCN			Clones low in HCN		
Clone number	HCN ppm	Picrate test*	Clone number	HCN ppm	Picrate test*
74-770	7.75	G+E	462	1.25	E
74-771	11.25	G+E	516c-4	0.75	E
74-772	15.25	G+E	516c-7	0.75	G
74-773	7.25	G+E	74-559-1	0.50	0
74-774	9.25	G+E	570n	0.75	0
74-776	5.25	G+E	628-4	0.25	E
74-777	8.75	G+E	74-639-7	4.00	G+E
74-778	12.25	G+E	715-8	1.00	E
74-779	10.00	G+E	715-9	1.00	0
74-781	12.00	G+E	715-11	0.75	G
74-782	13.00	G+E	716-7	0.50	0
74-783	12.00	G+E	719c-5	0.75	0
74-785	12.50	G+E	719c-7	0.50	G
74-786	11.50	G+E	720c-5	0.50	G
74-787	11.25	G+E	720c-6	0.50	G
74-788	8.00	G+E	720c-8	0.25	E
74-789	10.75	G+E	74-768	1.50	G+E
74-790	4.25	G+E	74-769	1.75	G+E
74-791	10.25	G+E	74-780	1.75	G
74-793	9.00	G+E	4384	1.25	G

* G+E, glucoside + enzyme (cyanogenic)
E, enzyme
G, glucosides
0, without both enzyme and glucosides.

Table 3. Clone designation, phenotypes, and results of serological tests with ATTC PVAS-62.

Clones high in HCN			Clones low in HCN		
Clone number	Phenotype	PSV*	Clone number	Phenotype	PSV*
74-770	G+E	+	462	E	
74-771	G+E		516c-4	E	
74-772	G+E	+	516c-7	G	
74-773	G+E	+	74-559-1	O	
74-774	G+E	+	470n	O	
74-776	G+E		628-4	E	+
74-777	G+E		74-639-7	G+E	
74-778	G+E	+	715-8	E	
74-779	G+E		715-9	O	
74-781	G+E	+	715-111	G	
74-782	G+E		716-7	O	
74-783	G+E	+	719c-5	O	
74-785	G+E	+	719c-7	G	+
74-786	G+E	+	720c-5	G	+
74-787	G+E		720c-6	G	+
74-788	G+E	+	720c-8	E	
74-789	G+E	+	74-768	G+E	
74-790	G+E	+	74-769	G+E	
74-791	G+E	+	74-780	G	
74-793	G+E		4384	G	

* + peanut stunt virus, positive reaction

Table 4. Relationship of glucosides x enzyme interaction on several characteristics of white clover.

		Enzyme		
Character	Glucosides	Absence	Presence	
Diameter of plants (cm)	Absence	40.32	49.90	*
	Presence	42.20	45.78	*
		ns	ns	
Height of plants (cm)	Absence	12.35	11.90	ns
	Presence	9.35	6.34	*
		*	*	
Length of stolon internode (cm)	Absence	1.918	2.285	*
	Presence	1.868	2.761	*
		ns	*	
Flowering time, (days after planting)	Absence	99.0	84.0	*
	Presence	96.1	44.8	*
		ns	*	
Life span, (days after planting)	Absence	187.4	184.8	ns
	Presence	182.1	171.1	*
		*	*	
Seed yield (g/plant)	Absence	8.27	8.04	ns
	Presence	4.44	4.93	ns
		*	*	

* significant at the 5% level. Asterisk to the right of a pair of values refers to absence or presence of enzymes, and asterisk below a pair refers to glucosides.

pair of means for one factor for every level of the second. Virus symptom rating was not included because the interaction was not significant. The virus rating was increased by the presence of glucosides and absence of enzyme.

The two genotypes with largest plant diameter means were Li_acac and Li_Ac_, while the tallest plants were found in the genotypes liliacac and Li_acac. Plants with longest stolon internodes had the Li_Ac_ and Li_acac genotypes, and the earliest flowering plants and those with the shortest life span were found to be Li_Ac_. The clones producing the greatest amount of seed had the genotypes liliacac and Li_acac. The use of supplemental light had a positive effect on abundance of flowering in the acyanogenic phenotypes, with consequent greater seed production. Gibson (1957) found that extended light period caused plants that normally flowered to reach a peak intensity of flowering earlier than usual and caused plants of non-flowering condition to blossom profusely. The great majority of the clones with the cyanogenic genotype (Li_Ac_) came from natural populations, and the acyanogenic genotypes (liliacac, Li_acac, and liliAc_), with some exceptions, came from introduced material being used in the breeding program. Nolin's Improved and Louisiana S-1, varieties often planted in Florida, were found to have relatively high levels of HCN. In these studies they are represented by the clones 74-791 and 74-790, respectively.

The results obtained in the polycross nursery indicated that the genotype Li_Ac_ (cyanogenic) is associated with a viny-type plant having large diameter, short stature, long stolon internodes, early flowering, and short life span. The acyanogenic genotypes with some exceptions were taller, bunch type plants, with shorter internodes and later flowering date. Gibson et al. (1963) found that plants of nonviny type were superior in persistence and forage production, compared with the viny type. They stated that selection for the nonviny type apparently is justified. Beinhart et al. (1963) observed that stolons of nonviny plants contributed greatly to increased leaf area and, presumably, to increased growth rate.

The differences among treatments (clones) within genotypes, determined by the Duncan's multiple range test, for the seven responses studied are shown in Tables 5 to 11, along with the treatment means.

There was a rather wide range of variation for the responses across genotypes; however, the relative magnitude of variation was not consistent according to genotypes. Cyanogenic plants (Li_Ac_) did flower over a shorter period of time than the acyanogenic genotypes. The life span of this group of plants was also more variable than the acyanogenic plants collectively.

The estimates of linear correlation separately for each genotype and for six responses studied are shown in Table 12. The diameter of the plants was positively correlated with height and length of stolon internodes of the plants for all genotypes. Within genotypes the plants with large diameter were associated with tall plants and with long stolon itnernodes. Flowering time was negatively correlated with length of stolon internodes and seed yield in genotypes Li_acac and liliAc_. Within genotypes, generally the plants that flowered late had short stolon internodes and produced few seeds. Gibson and Hollowell (1966) pointed out that each flower head reduces the potential branch stolons by one, and branching during periods of profuse flowering is greatly reduced or not present. The life span of the plants was positively correlated with diameter, height, and flower time in genotypes

Table 5. Average plant diameter according to genotype.

Genotype liliacac

Clone number	Average diameter (cm)	
716-7	62.25	a *
719c-5	38.25	b
74-559-1	37.62	b
570n	34.75	b
715-9	28.75	b

Genotype Li_acac

Clone number	Average diameter (cm)	
720c-8	58.75	a
516c-4	56.00	ab
628-4	45.88	bc
462	44.88	c
715-8	44.00	c

Genotype liliAc_

Clone number	Average diameter (cm)	
516c-7	62.50	a
74-780	54.75	ab
4384	50.25	b
715-11	44.75	b
719c-7	33.25	c
720c-6	29.12	cd
720c-5	20.75	d

Genotype Li_Ac_

Clone number	Average diameter (cm)	
74-769	62.25	a
74-639-7	58.38	ab
74-790	56.88	ab
75-779	56.00	ab
74-791	54.50	abc
74-788	52.00	abcd
74-786	50.75	abcde
74-787	49.25	bcde
75-777	48.88	bcde
74-772	46.62	bcde
74-793	46.50	bcde
74-789	43.25	cdef
74-783	42.75	cdef
74-774	42.50	cdef
74-776	42.00	def
74-771	41.75	def
74-782	41.25	def
74-781	41.00	def
74-785	39.62	efg
74-770	37.62	fg
74-778	37.00	fg
74-768	33.62	fg
74-773	28.62	g

* Means followed by the same letter are not significantly different at the 5% level.

Table 6. Average plant height according to genotype.

Genotype liliacac	
Clone number	Average height (cm)
74-559-1	17.25 a *
716-7	13.50 b
719c-5	13.50 b
570n	9.19 c
715-9	8.31 c
Genotype Li_acac	
516c-4	14.12 a
462	13.00 ab
720c-8	12.12 b
628-4	11.50 b
714-8	8.75 c
Genotype liliAc_	
4384	16.25 a
516c-7	12.06 b
715-11	8.88 c
719c-7	8.25 c
74-780	8.00 cd
720c-5	6.31 de
720c-6	5.69 e

Genotype Li_Ac_	
Clone number	Average height (cm)
74-639-7	9.88 a
74-771	9.12 ab
74-769	9.12 ab
74-791	8.69 abc
74-783	7.88 bcd
74-787	7.19 bcde
74-790	6.81 cde
74-770	6.81 cde
74-788	6.69 def
74-773	6.56 def
74-776	6.38 defg
74-785	5.88 defgh
74-772	5.88 defgh
74-768	5.88 defgh
74-777	5.75 efghi
74-779	5.56 efghij
74-793	5.44 efghij
74-786	5.31 efghij
74-789	4.71 fghij
74-774	4.44 ghij
74-778	4.25 hij
74-782	3.81 ij
74-781	3.69 j

* Means followed by the same letter are not significantly different at the 5% level.

Table 7. Average stolon internode length according to genotype.

Genotype liliacac

Clone number	Average length (cm)	
716-7	2.962	a *
719c-5	1.988	b
715-9	1.588	b
570n	1.538	b
74-559-1	1.512	b

Genotype Li_acac

Clone number	Average length (cm)	
516c-4	3.100	a
720c-8	2.250	b
628-4	2.238	b
715-8	1.938	b
462	1.900	b

Genotype liliAc_

Clone number	Average length (cm)	
516c-7	2.888	a
74-780	2.638	ab
715-11	2.175	b
4384	1.575	c
720c-5	1.288	c
720c-6	1.275	c
719c-7	1.238	c

Genotype Li_Ac_

Clone number	Average length (cm)	
74-769	4.038	a
74-790	3.612	ab
74-786	3.475	abc
74-793	3.325	bcd
74-778	3.115	bcde
74-787	3.075	bcde
74-788	3.050	bcde
74-789	2.962	cde
74-779	2.950	cdef
74-785	2.900	cdefg
74-791	2.825	defg
74-772	2.775	defg
74-777	2.750	defgh
74-782	2.662	efghi
74-781	2.588	efghi
74-783	2.575	efghi
74-639-7	2.538	efghi
74-771	2.312	fghij
74-774	2.288	ghij
74-773	2.125	hij
74-768	2.062	ij
74-770	1.812	j
74-776	1.700	j

* Means followed by the same letter are not significantly different at the 5% level.

Table 8. Average virus symptom rating according to genotype.

Genotype liliacac

Clone number	Average (index)	
719c-5	1.875	a *
570n	1.500	ab
74-559-1	1.000	ab
715-9	1.000	ab
716-7	0.625	b

Genotype Li_acac

Clone number	Average (index)	
715-8	2.625	a
516c-4	0.500	b
628-4	0.250	b
720c-8	0.125	b
462	0.000	b

Genotype liliAc_

Clone number	Average (index)	
719c-7	4.000	a
720c-6	3.000	a
720c-5	1.750	b
715-11	0.875	bc
74-780	0.625	c
516c-7	0.500	c
4384	0.000	c

Genotype Li_Ac_

Clone number	Average (index)	
74-770	2.875	a
74-778	2.875	a
74-781	2.375	ab
74-773	2.125	abc
74-771	1.750	bcd
74-779	1.500	bcde
74-788	1.250	cdef
74-772	1.125	cdefg
74-785	1.000	cdefg
74-793	1.000	cdefg
74-783	0.875	defg
74-774	0.750	defg
74-776	0.625	defg
74-790	0.625	defg
74-789	0.500	efg
74-639-7	0.500	efg
74-782	0.500	efg
74-777	0.375	efg
74-787	0.375	efg
74-786	0.250	fg
74-768	0.250	fg
74-791	0.125	fg
74-769	0.000	g

* Means followed by the same letter are not significantly different at the 5% level.

Table 9. Average days to flowering according to genotype.

Genotype liliacac			Genotype Li_Ac_		
Clone number	Average (days)		Clone number	Average (days)	
570n	133.4	a *	74-774	86.7	a
719c-5	127.2	a	74-789	71.1	b
716-7	90.6	b	74-768	63.1	bc
74-559-1	84.2	b	74-793	62.5	bc
715-9	66.8	c	74-778	59.5	bcd
			74-771	54.0	cde
Genotype Li_acac			74-787	53.8	cde
462	117.2	a	74-781	53.2	cde
720c-8	113.8	a	74-791	48.6	cdef
715-8	82.7	b	74-639-7	48.5	cdef
628-4	55.6	c	74-785	46.1	def
516c-4	50.4	c	74-790	45.6	def
			74-772	45.5	def
Genotype liliAc_			74-779	41.6	efg
4384	133.7	a	74-783	36.9	fgh
715-11	129.0	a	74-786	34.4	fgh
719c-7	122.4	a	74-782	32.6	fghi
720c-5	119.2	a	74-769	29.5	ghi
720c-6	81.5	b	74-770	28.1	ghi
516c-7	60.0	c	74-788	27.8	ghi
74-780	37.5	d	74-776	25.4	ghi
			74-777	23.0	hi
			74-773	17.5	i

* Means followed by the same letter are not significantly different at the 5% level.

Table 10. Average life span according to genotype.

Genotype liliacac

Clone number	Average (days)	
74-559-1	190.9	a *
570n	188.8	a
719c-5	187.8	a
716-7	187.2	a
715-9	182.1	a

Genotype Li_acac

Clone number	Average (days)	
462	199.1	a
720c-8	187.2	ab
516c-4	183.9	bc
628-4	181.8	bc
715-8	172.1	c

Genotype liliAc_

Clone number	Average (days)	
4384	192.0	a
516c-7	187.2	ab
719c-7	184.0	abc
715-11	183.9	abc
74-780	179.6	abc
720c-5	177.0	bc
720c-6	171.1	c

Genotype Li_Ac_

Clone number	Average (days)	
74-639-7	189.0	a
74-789	181.8	ab
74-768	179.8	abc
74-774	179.4	abc
74-771	178.6	abc
74-793	175.9	abcd
74-787	175.6	abcd
74-769	175.1	abcd
74-777	173.5	bcd
74-790	173.5	bcd
74-776	173.1	bcd
74-779	173.0	bcd
74-788	172.0	bcd
74-791	170.4	bcde
74-783	169.4	bcdef
74-782	167.9	bcdef
74-770	165.8	cdef
74-781	163.2	def
74-785	163.2	def
74-778	162.0	def
74-786	161.1	def
74-773	155.9	ef
74-772	155.4	f

*Means followed by the same letter are not significantly different at the 5% level.

Table 11. Average seed yield according to genotype.

Genotype liliacac			Genotype Li_Ac_		
Clone number	Average (g)		Clone number	Average (g)	
716-7	18.36	a *	74-639-7	16.60	a
74-559-1	14.14	b	74-771	10.48	b
570n	4.51	c	74-791	10.00	bc
715-9	2.30	c	74-776	6.62	bcd
719c-5	2.03	c	74-789	5.64	cde
			74-783	5.46	de
Genotype Li_acac			74-790	5.33	de
516c-4	20.30	a	74-769	5.19	de
628-4	7.99	b	74-785	5.12	de
720c-8	5.06	bc	74-788	4.86	de
462	3.94	bc	74-772	4.72	de
715-8	2.89	c	74-777	4.32	de
			74-787	3.96	de
Genotype liliAc_			74-770	3.76	de
516c-7	13.87	a	74-781	3.56	de
74-780	7.35	a	74-782	3.19	de
4384	3.38	b	74-779	3.13	de
719c-7	2.50	bc	74-774	2.80	de
715-11	2.47	c	74-786	2.76	de
720c-5	1.25	c	74-773	1.89	de
720c-6	0.22	c	74-793	1.82	de
			74-778	1.43	e
			74-768	0.87	e

* Means followed by the same letter are not significantly different at the 5% level.

Table 12. Correlation coefficients for six responses studied in the polycross nursery.

		Responses				
Genotype	Responses	Height of pl. (cm)	Length of int. (cm)	Flower time (days)	Life span (days)	Seed yield (g)
liliacac	Diameter	.4729**	.8714**	-.0063	.1388	.6605**
	Height		.2875	-.0864	.2443	.5060**
	Length			-.0434	.0347	.4594**
	Flower				.0769	-.1286
	Life					.2320
Li_acac	Diameter	.5638**	.5565**	-.0814	.2052	.4757**
	Height		.5025**	-.0710	.4141**	.5971**
	Length			-.5589**	.1273	.7208**
	Flower				.4437**	-.5231**
	Life					-.1552
liliAc_	Diameter	.5852**	.7595**	-.4978**	.3376**	.7415**
	Height		.2772*	-.1680	.4760**	.2959*
	Length			-.6436**	.1545	.5930**
	Flower				.3065*	-.5463**
	Life					.2004
Li_Ac_	Diameter	.4981**	.5696**	-.1002	.3033**	.5124**
	Height		.2048**	-.1751*	.2966**	.5966**
	Length			.0452	.1092	.0861
	Flower				.1530*	-.0592
	Life					.4401*

* Significant at the 5% level.
** Significant at the 1% level.

liliAc_ and Li_Ac_, and with height and flower time in genotype Li_acac. Generally for each genotype the plants that survived longer were those with large diameter, which were tall and remained vegetative for a longer period of time. The seed yield for all genotypes was generally positively associated with diameter of the plants, height, and length of stolon internodes. In the genotype Li_Ac_ the plants that survived longer generally produced more seed than the plants with short life span.

Progeny Test

The results of the measurements made on the progenies with respect to the responses studied, along with the analysis of variance, are shown in the Appendices A-11 to A-18.

With respect to the factors glucosides and enzyme, few significant differences were found in their progenies. The interaction between enzyme and glucosides was significant for both flowering time and HCN content. The nature of these interactions is shown in Table 13. Included are the interaction means along with the results of the t-test performed on each pair of means for one factor at every level of the second. The progenies from cyanogenic clones flowered earlier than the progenies from clones with only glucosides, and these progenies did not differ from the progenies of the other two genotypes with respect to this response. The HCN content was higher in the progenies from cyanogenic clones followed by the progenies of the clones with only glucosides. The differences among progenies within genotypes, determined by the Duncan's multiple range test for these two responses, are shown in Table 14.

The length of stolon internodes was affected by the presence of

Table 13. Relationship of glucosides x enzyme interaction on characteristics of white clover progenies.

		Enzyme	
Character	Glucosides	Absence	Presence
Flowering time (days after planting)	Absence	138.5	138.4 ns
	Presence	149.2	132.5 *
		ns	ns
HCN content (ppm)	Absence	1.22	1.68ns
	Presence	1.26	2.79*
		*	*

* Significant at the 5% level.

Table 14. Average flowering time and HCN content according to progenies of three genotypes.

Genotype liliAc_	
Progeny number	Average flowering, days
516c-7	196.2 a *
719c-7	144.8 b
720c-5	143.0 b
715-11	141.8 b
4384	141.5 b
74-780	128.0 b

Progeny number	Average HCN cont., ppm
516c-4	2.88 a
720c-8	1.75 ab
715-8	1.56 b
628-4	1.31 b
462	0.88 b

Genotype Li_Ac_	
Progeny number	Average HCN content, ppm
74-782	4.44 a
74-773	4.00 ab
74-787	3.88 abc
74-779	3.50 abcd
74-772	3.38 abcde
74-774	3.38 abcde
74-776	3.25 abcde
74-783	3.19 abcde
74-777	3.06 abcdef
74-791	3.00 abcdef
74-771	2.75 bcdefg
74-785	2.69 bcdefg
74-770	2.56 bcdefg
74-788	2.44 cdefg
74-786	2.38 cdefg
74-793	2.38 cdefg
74-790	2.12 defg
74-639-7	2.00 defg
74-789	1.94 efg
74-781	1.88 efg
74-778	1.62 fg
74-769	1.50 g

* Means followed by the same letter are not significantly different at the 5% level.

the enzyme. The progenies from genotypes Li_Ac_ and Li_acac had longer stolon internodes than those from the other genotypes (Figure 5). The results of the dry matter yield on the first, second, and third harvests, and total are shown in Figures 1, 2, 3, and 4 respectively. There was a tendency of the progenies from the clones with more dominant genes to produce more clover dry matter yield on the first harvest, but the differences for both grasses and clover among and within genotypes were not significant. On the second and third harvests this tendency appeared to change in the opposite direction, the progenies with less dominant genes producing more white clover dry matter yield, although the differences observed on these harvests and in the total clover dry matter yield were not significant.

The polycross method, as was pointed out by Graumann (1952), is a very efficient breeding method for cross pollinated species that possess small flowers. In this particular work it was very efficient in reducing differences among progenies of different clone genotypes. Figure 6 shows the amount of HCN (ppm) in the clones selected and in their respective progenies. The variation of the HCN content of the clones among genotypes was not observed to the same extent among the progenies. The length of stolon internodes increased proportionally in the progenies of all genotypes, compared with the length of stolon internode of the parent clones (Figure 5).

The ranges of variation in flowering time among genotypes of individual plants and their progenies are shown in Figure 7. With the exception of genotype liliAc_, which had one late flowering progeny, all the progenies showed a reduction in the flowering period relative to the parental clones.

The summary of the results obtained in the progeny test is shown in Table 15.

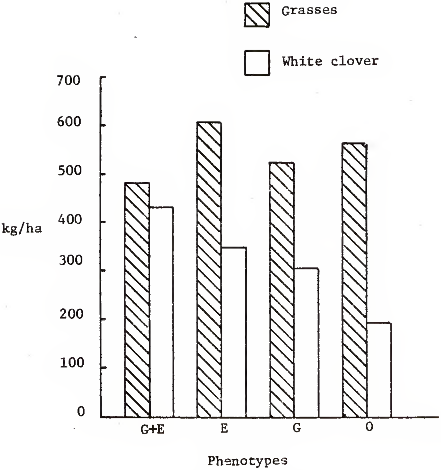

Fig. 1. Average dry matter yield on the first harvest (4/13/76) according to phenotype.

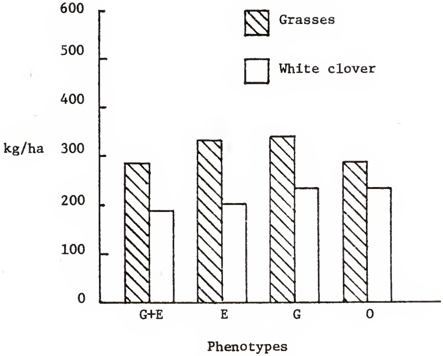

Fig. 2. Average dry matter yield on the second harvest (5/11/76) according to phenotype.

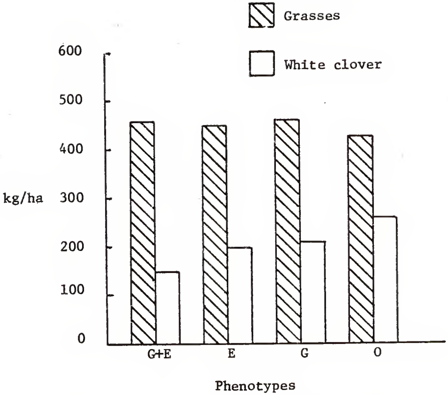

Fig. 3. Average dry matter yield on the third harvest (6/8/76) according to phenotype.

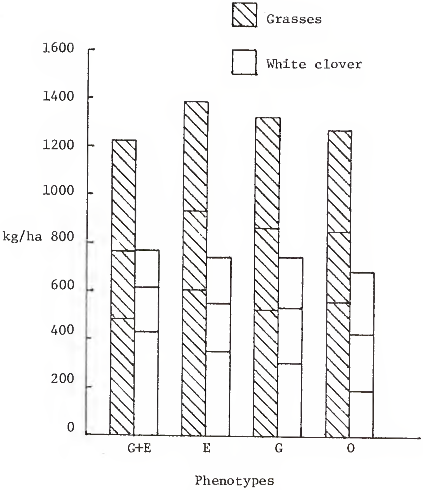

Fig. 4. Average total dry matter yield during the experimental period (horizontal bars separate the three harvests).

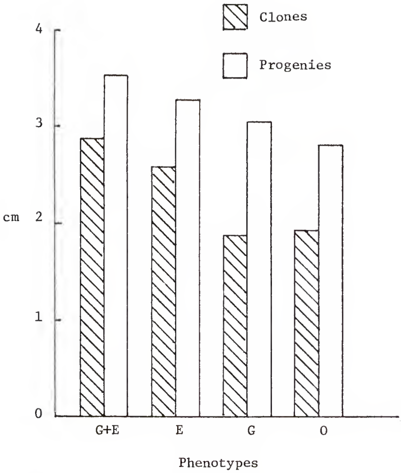

Fig. 5. Average length of stolon internodes according to phenotypes of the clones and their respective progenies.

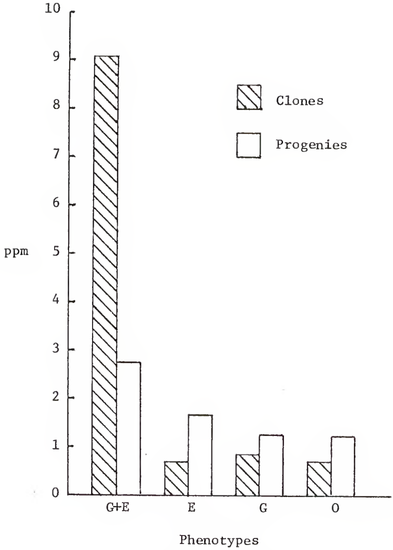

Fig. 6. Average HCN content according to phenotypes of the clones and their respective progenies.

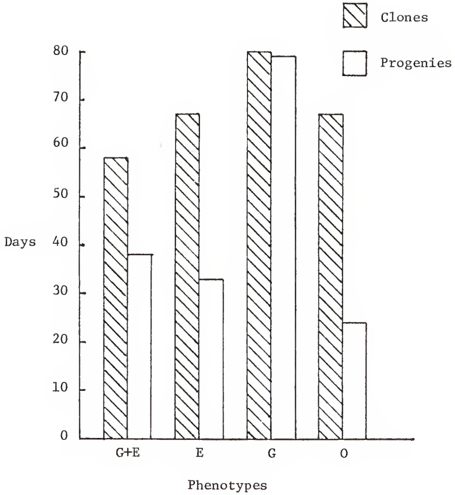

Fig. 7. Ranges of variation in flowering time according to phenotypes of the clones and their respective progenies.

Table 15. Summary of the results obtained in the progeny test, 1976.*

Genotypes	HCN ppm	Flowering time, days	Length of stolon int. cm	Total clover yield kg/ha
Li_Ac_	2.79	132	3.54	678
Li_acac	1.68	138	3.26	630
liliAc_	1.26	149	3.03	642
liliacac	1.22	138	2.81	637

* Average over genotypes.

The results obtained in this experiment indicated that the use of cyanogenic plants in development and adaptation of varieties, including the Ladino type, to warm conditions might be very useful. The HCN content was reduced in the progenies which resulted from intercrossing acyanogenic and cyanogenic plants. The progenies also had an average flowering time varying from 132 to 149 days after planting, which represents a short interval of only 17 days. This situation should certainly insure more uniform seed production. The variation in length of stolon internodes among progenies within genotypes was not great, and the dry matter yields of the cyanogenic anc acyanogenic progenies were not significantly different.

SUMMARY AND CONCLUSIONS

The high frequency of cyanogenic plants of white clover (*Trifolium repens* L.) in warm conditions is frequently reported. Cyanogenesis is also reported as a mechanism of defense against herbivores. The purpose of this study was to survey plants occurring in natural populations and introduced breeding lines for the presence and absence of HCN and observe their relationship with other characteristics, in individual plants and their progenies after intercrossing in a polycross nursery.

The content of HCN of plants occurring in natural populations and introduced breeding lines was analyzed by the alkaline picrate solution method in 1974. Two groups of 20 clones, one considered high in HCN and the other considered low in HCN, were separated and maintained in the greenhouse. Later the picrate test was used to separate them into the four possible genotypes for the presence and absence of the glucosides and/or enzyme. The results indicated that 23 clones had the Li_Ac_ (G+E), 5 had liliacac (O), 5 had Li_acac (E), and 7 had liliAc_ (G) genotypes. The majority of the cyanogenic type (G+E) was from natural populations, while most of the acyanogenic material was from introduced breeding lines. Virus symptoms were observed in the material maintained in the greenhouse, and serological tests with ATCC PVAS 62 antiserum indicated the presence of peanut stunt virus in several clones.

The 40 clones replicated eight times were planted in a polycross nursery. The following measurements were made: diameter of the plants in cm, height of the plants in cm, length of stolon internode in cm, virus symptom rating according to the scale 0 to 4 from absence to very strong symptoms, flowering time from the date of the first open flower and computed as days after planting, life span of the plants computed in days after planting, and seed yield in grams per plant. With respect to the factors glucosides and enzyme, all responses showed significant effects. Except for the virus symptom rating, the effects of these two factors were found to interact. The results obtained in the polycross nursery indicated that the genotype Li_Ac_ (cyanogenic) is associated with a viny type plant having large diameter, short stature, long stolon internodes, early flowering, and short life span. The acyanogenic genotypes (liliacac, Li_acac and liliAc_) with some exceptions were taller, bunch type plants, with shorter internodes and later flowering date. There was a rather wide range of variation for the responses across genotypes; however, the relative magnitude of variation was not consistent according to genotypes. Cyanogenic plants (Li_Ac_) did flower over a shorter period of time than the acyanogenic genotypes. The life span of this group of plants was more variable than the acyanogenic plants collectively.

Linear correlation was estimated separately for each genotype, for the six responses studied. Most characters showed positive correlation among themselves; however, a few negative correlations were noted.

A progeny test was conducted with 38 clones that produced enough seed to plant plots 1.8 x 1.8 m with four replications in a randomized complete-block design. The area was covered with grasses which were mowed and fertilized followed by scarification with a disk. The seeds were broadcast on the surface of the plots and irrigation was used to facilitate germination and establishment of the seedlings. A period of low temperature occurred in December of 1975, causing damage to the seedlings and reducing the stand on the majority of the plots. The following measurements were made on the experimental material: HCN content, flowering time in days after planting, length of stolon internodes in cm, and dry matter yield in kg/ha. For the estimation of the dry matter yield an area of 1.35 m^2 was harvested in each plot. The green material was weighed, and samples were taken for determination of dry matter and separation of the components, grasses and white clover.

With respect to the factors glucosides and enzyme, few significant differences were found among the progenies. For both flowering time and HCN content, the interaction between enzyme and glucosides was significant. The progenies from cyanogenic clones flowered earlier than the progenies from clones with only glucosides, and these progenies do not differ from the progenies of the other two genotypes. The HCN content was higher in the progenies from cyanogenic clones followed by the progenies of the clones with only glucosides. The length of stolon internodes was associated with the presence of the enzyme; the progenies of genotypes Li_Ac_ and Li_acac had longer stolon internodes. Over the three harvests performed, the differences in white clover dry matter yield among and within genotypes were not significant.

The correlations and associations of characteristics of white clover observed in this study might be very useful to the plant breeder in selection and improvement of varieties in the future.

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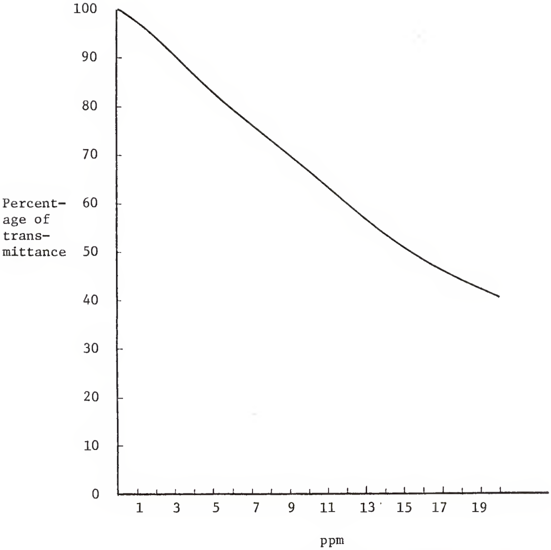
A-1. Clone designation and percent of transmittance, at 575 mμ, of the clones and their respective progenies.

Clone number	Clones (green house)	Progenies*			
		I	II	III	IV
74-770	74.5	92.3	92.5	91.0	93.5
74-771	62.5	95.0	88.0	91.0	93.5
74-772	50.2	92.0	81.5	92.0	92.5
74-773	76.0	86.8	90.0	87.0	83.5
74-774	69.5	80.0	89.5	93.0	95.5
74-776	82.5	90.0	90.0	87.5	92.5
74-777	70.5	95.0	90.5	87.0	89.5
74-778	59.5	97.0	96.0	94.0	95.0
74-779	66.5	93.0	80.0	92.0	92.0
74-781	60.0	95.0	94.0	94.5	96.0
74-782	56.5	75.0	87.5	91.5	89.0
74-783	60.0	83.5	94.0	86.5	96.5
74-785	58.5	91.0	92.0	94.5	91.0
74-786	62.0	91.5	93.5	93.5	93.5
74-787	63.5	81.0	85.0	91.5	91.5
74-788	73.2	86.0	95.0	94.0	95.5
74-789	64.0	96.0	94.7	91.0	97.0
74-790	96.5	93.0	94.5	94.0	94.0
74-791	65.5	91.5	93.5	89.5	89.0
74-793	70.0	95.5	95.0	91.5	90.0
462	97.5	98.5	99.0	98.5	96.5
516c-4	98.5	81.5	94.5	92.5	96.0
516c-7	98.5	99.0	98.0	98.0	96.5
74-559-1	99.0	96.5	98.0	96.5	97.5
570n	98.5	98.0	99.0	97.5	97.5
628-4	99.5	95.5	97.5	96.0	98.5
74-639-7	88.0	93.5	93.5	96.0	94.0
715-8	98.0	95.0	97.0	94.5	97.5
715-9	98.0	97.0	96.0	95.5	96.0
715-11	98.5	95.5	97.5	95.5	97.5
716-7	99.0	98.0	98.0	96.5	97.5
719c-5	98.5	98.0	96.5	97.0	98.5
719c-7	99.0	99.0	98.5	95.5	96.5
720c-5	99.0	97.0	99.0	97.0	96.5
720c-6	99.0				
720c-8	99.5	88.5	98.0	98.0	97.5
74-768	96.5				
74-769	95.5	97.0	95.0	90.0	93.0
74-780	95.7	95.0	94.5	96.5	97.0
4384	97.5	98.0	97.5	97.5	97.5

* Replications of the progeny test.



A-2. Standard curve for estimation of the amount of HCN (575 mμ).

A-3. Diameter of plants in cm, February 22, 1975.

Clones	Replications							
	1	2	3	4	5	6	7	8
74-770	30	46	32	40	40	33	40	40
74-771	44	53	37	38	44	43	35	40
74-772	54	57	44	60	50	43	35	30
74-773	25	15	25	36	26	30	22	50
74-774	40	40	48	56	4	44	48	60
75-776	30	40	46	50	45	41	44	40
74-777	40	45	58	60	48	40	55	45
74-778	36	38	16	44	38	52	42	30
74-779	59	50	62	60	54	30	73	60
74-781	20	38	36	36	75	35	48	40
74-782	36	46	21	48	42	40	42	55
74-783	40	54	40	46	48	31	48	35
74-785	12	56	66	32	35	38	48	30
74-786	38	63	60	30	38	55	62	60
75-787	42	48	64	21	36	68	65	50
74-788	40	53	60	50	55	54	54	50
74-789	38	42	45	44	37	60	45	35
74-790	30	54	70	44	70	50	82	55
74-791	64	65	53	55	59	55	72	13
74-793	42	50	64	50	33	35	58	40
362	43	40	50	40	38	48	50	50
516c-4	43	60	62	60	30	69	64	60
516c-7	36	70	76	65	75	34	84	60
74-559-1	37	40	42	42	28	32	45	35
570n	36	32	42	24	47	37	25	35
628-4	43	46	47	51	50	47	48	35
74-639-7	50	53	54	63	63	50	64	70
715-8	36	54	28	53	44	45	52	40
715-9	30	27	34	23	33	26	27	30
715-11	50	44	49	50	50	35	40	40
716-7	60	68	67	58	58	67	60	60
719c-5	20	35	40	46	38	32	50	45
719c-7	30	34	43	26	42	31	36	24
720c-5	10	25	14	17	24	36	15	25
720c-6	40	22	8	32	16	45	35	35
720c-8	60	50	60	52	63	48	82	55
74-768	24	38	26	32	38	40	38	33
74-769	50	68	74	37	56	55	73	85
74-780	45	50	49	55	53	57	59	70
4384	38	50	50	47	50	57	60	50

A-4. Height of plants in cm, February 22, 1975.

Clones	Replications							
	1	2	3	4	5	6	7	8
74-770	5.0	6.0	8.0	7.5	8.0	4.0	8.0	8.0
74-771	8.0	9.0	9.0	9.0	8.0	10.0	8.0	12.0
74-772	5.0	5.0	5.0	7.0	6.0	6.0	8.0	5.0
74-773	5.5	5.0	5.0	9.0	8.0	8.0	3.0	9.0
74-774	3.0	3.0	6.0	5.0	1.5	6.0	5.0	6.0
74-776	4.0	6.0	7.0	8.0	8.0	6.0	7.0	5.0
74-777	5.0	4.0	8.0	5.0	6.0	4.0	9.0	5.0
74-778	3.0	4.5	2.5	4.0	5.0	6.0	4.0	5.0
74-779	6.0	7.0	4.0	6.5	6.0	4.5	4.5	6.0
74-781	1.5	3.0	6.0	2.5	6.0	3.5	4.0	3.0
74-782	3.5	5.0	4.0	4.0	4.0	2.0	3.0	5.0
74-783	8.0	8.0	8.0	10.0	7.0	7.0	9.0	6.0
74-785	3.0	11.0	7.0	4.0	4.0	6.0	6.0	6.0
74-786	3.0	6.0	6.0	6.5	5.0	4.0	4.0	8.0
74-787	5.0	9.0	9.5	3.0	5.0	9.0	8.0	9.0
74-788	5.0	8.0	7.0	6.0	7.0	6.5	9.0	5.0
74-789	5.0	6.0	4.0	5.5	3.2	6.5	3.5	4.0
74-790	4.5	9.0	7.0	4.0	8.0	7.0	5.0	10.0
74-791	11.0	10.0	6.5	9.5	8.0	9.0	11.0	4.5
74-793	4.5	7.0	5.5	6.0	2.0	3.5	8.0	7.0
462	12.0	13.0	14.0	12.0	11.0	14.0	14.0	14.0
516c-4	9.0	16.0	16.0	16.0	11.0	13.0	16.0	16.0
516c-7	11.0	13.0	13.0	13.5	11.0	8.0	15.0	12.0
74-559-1	15.0	19.0	18.0	16.0	16.0	18.0	18.0	18.0
570n	9.0	8.0	14.0	5.5	11.0	9.0	7.0	10.0
628-4	12.0	10.0	13.0	12.0	12.0	12.0	10.0	11.0
74-639-7	8.0	10.0	10.0	11.0	13.0	7.0	10.0	10.0
715-8	9.5	10.0	6.0	10.0	9.0	9.0	9.0	7.5
715-9	8.0	8.5	9.0	7.0	11.0	7.0	7.0	9.0
715-11	11.0	9.0	14.0	9.0	2.0	9.0	8.0	9.0
716-7	14.0	14.0	14.0	14.0	10.0	14.0	14.0	14.0
719c-5	8.0	14.0	14.0	17.0	13.0	13.0	14.0	15.0
719c-7	6.0	9.0	9.0	7.0	10.0	9.0	10.0	6.0
720c-5	3.5	11.0	4.0	5.0	6.0	8.0	6.0	7.0
720c-6	5.0	4.5	5.0	7.0	3.5	6.0	5.5	9.0
720c-8	13.0	12.0	13.0	13.0	13.0	10.0	12.0	11.0
74-768	4.5	5.0	6.0	7.5	5.5	7.0	5.5	6.0
74-769	12.0	9.0	11.0	6.0	9.0	8.0	9.0	9.0
74-780	9.0	9.0	9.0	6.5	8.0	5.5	10.0	7.0
4384	13.0	17.0	15.0	16.0	18.0	19.0	16.0	16.0

A-5. Length of stolon internode in cm, February 22, 1975.

Clones	Replications 1	2	3	4	5	6	7	8
74-770	2.0	1.2	1.5	2.2	1.8	1.8	1.8	2.2
74-771	2.7	3.0	2.2	2.2	2.2	2.5	1.2	2.5
74-772	2.0	3.0	2.8	2.6	3.5	2.8	3.0	2.5
74-773	2.0	0.5	1.5	2.3	2.5	3.1	1.8	3.3
74-774	2.0	1.5	2.5	2.0	0.2	2.6	2.5	5.0
74-776	2.0	1.7	2.1	1.5	2.0	1.8	1.0	1.5
74-777	3.0	2.7	2.7	2.3	2.6	2.7	3.0	3.0
74-778	3.5	3.0	2.5	2.7	2.5	5.0	3.5	2.2
74-779	3.2	2.8	3.5	2.5	3.7	1.9	3.0	3.0
74-781	2.5	2.3	2.7	2.5	3.5	1.7	2.5	3.0
74-782	3.0	2.2	2.0	3.0	2.9	2.2	2.5	3.5
74-783	2.2	3.0	2.7	2.5	2.7	2.0	2.8	2.7
74-785	1.0	2.7	3.0	3.0	4.0	3.8	3.5	2.2
74-786	4.0	3.4	4.0	3.0	2.9	3.0	3.5	4.0
74-787	3.2	3.0	3.5	1.2	3.2	4.0	3.0	3.5
74-788	2.4	2.8	3.3	2.5	4.5	2.8	3.4	2.7
74-789	3.0	1.7	3.7	3.7	3.2	2.5	2.5	3.4
74-790	3.4	3.2	4.0	3.0	3.7	3.5	4.3	3.8
74-791	3.0	3.2	3.0	2.9	2.3	3.8	3.2	1.2
74-793	3.0	3.8	3.0	3.2	2.7	4.2	3.5	3.2
462	2.0	1.8	2.0	1.9	2.0	1.8	1.7	2.0
516c-4	3.2	3.7	3.7	3.2	2.4	2.9	3.2	2.5
516c-7	2.5	2.7	2.5	3.0	3.5	2.5	3.2	3.2
74-559-1	1.7	1.5	1.2	1.5	1.2	1.5	2.0	1.5
570n	1.6	1.6	1.7	1.1	1.8	1.5	1.2	1.8
628-4	2.2	2.4	2.6	2.5	2.0	2.2	2.2	1.8
74-639-7	2.3	2.7	2.9	2.2	2.5	2.5	2.2	3.0
715-8	1.6	2.5	1.5	3.0	2.0	1.2	1.9	1.8
715-9	1.5	1.4	2.1	1.0	2.0	1.5	1.3	1.9
715-11	1.9	2.5	2.0	2.0	2.0	2.0	2.5	2.5
716-7	2.8	3.0	3.7	3.2	3.0	2.8	2.5	2.7
719c-5	1.5	2.1	2.1	2.8	1.7	1.5	2.2	2.0
719c-7	1.0	1.0	1.5	1.2	1.4	1.2	1.2	1.4
720c-5	0.8	1.9	1.0	0.9	1.7	1.5	1.0	1.5
720c-6	1.7	1.2	0.5	1.4	1.0	1.0	1.4	2.0
720c-8	2.2	1.7	2.0	2.5	2.5	2.0	2.6	2.5
74-768	1.5	2.2	2.7	3.4	2.0	1.2	2.0	1.5
74-769	3.7	4.2	4.8	3.1	4.0	4.0	4.5	4.0
74-780	2.5	2.0	2.5	2.7	3.2	3.0	2.5	2.7
4384	1.2	1.0	1.5	1.7	1.5	2.2	1.8	1.7

A-6. Virus symptom index, polycross nursery, 1975.

Clones	Replications							
	1	2	3	4	5	6	7	8
74-770	2	4	3	2	3	3	4	2
74-771	0	1	2	1	0	2	4	4
74-772	1	1	3	2	0	2	0	0
74-773	3	1	4	1	3	2	1	2
74-774	0	4	1	0	0	1	0	0
74-776	0	1	0	2	1	2	0	0
74-777	1	0	1	0	0	0	0	0
74-778	3	2	2	4	4	3	3	2
74-779	0	1	3	3	1	1	3	0
74-781	0	3	3	4	2	1	4	2
74-782	0	2	1	0	1	0	0	0
74-783	2	2	0	1	0	2	0	0
74-785	2	0	0	1	0	1	2	2
74-786	0	1	0	0	1	0	0	0
74-787	0	0	0	3	0	0	0	0
74-788	3	0	3	2	2	0	0	0
74-789	0	1	0	1	0	1	1	0
74-790	0	0	1	0	0	0	0	4
74-791	0	0	0	0	0	0	0	1
74-793	0	0	0	0	0	4	4	0
462	0	0	0	0	0	0	0	0
516c-4	1	0	0	0	1	0	0	2
516c-7	0	2	0	0	0	0	0	2
74-559-1	2	1	1	0	1	1	2	0
570n	1	1	1	4	1	1	0	3
628-4	0	0	0	0	0	1	1	0
74-639-7	0	0	0	0	0	4	0	0
715-8	1	1	3	2	4	4	3	3
715-9	0	2	1	2	0	2	1	0
715-11	0	1	1	1	1	2	1	0
716-7	0	0	1	2	1	1	0	0
719c-5	1	4	0	1	3	4	2	0
719c-7	4	4	4	4	4	4	4	4
720c-5	0	0	2	4	0	4	2	2
720c-6	4	3	2	3	2	4	3	3
720c-8	0	0	0	0	0	1	0	0
74-768	0	0	0	0	1	1	0	0
74-769	0	0	0	0	0	0	0	0
74-780	0	0	4	0	0	0	1	0
4384	0	0	0	0	0	0	0	0

A-7. Flowering time in days after planting, polycross nursery, 1975.

Clones	Replications							
	1	2	3	4	5	6	7	8
74-770	29	38	31	24	24	24	31	24
74-771	57	48	48	62	57	62	48	60
74-772	41	45	43	43	48	48	48	48
74-773	2	10	14	22	38	22	10	22
74-774	85	73	83	113		85	90	78
74-776	13	31	24	27	22	31	31	24
74-777	18	13	10	47	57	2	27	10
74-778	57	64	59	59	57	57	64	59
74-779	50	24	48	50	55	31	48	27
74-781	66	48	59	48	43	62	52	48
74-782	59	24	66	22	38	2	28	22
74-783	10	34	41	38	38	48	43	43
74-785	43	45	10	52	57	57	48	57
74-786	34	38	22	36	48	36	27	34
74-787	57	55	48	62	62	48	50	48
74-788	55	10	55	18	18	29	6	31
74-789	78	66	69	69	69	62	71	85
74-790	59	48	52	62	18	10	57	59
74-791	27	52	52	57	27	55	55	64
74-793	62	59	59	62	64	69	59	66
462	113	157	115	133	115	113	101	111
516c-4	45	45	48	48	57	62	50	48
516c-7	64	62	50	62	62	64	57	59
74-559-1	134	113	64	62	64	62	113	62
570n	136	129	122	134	115	141	154	136
628-4	55	62	59	57	62	62	24	64
74-639-7	55	55	48	55	55	48	50	22
715-8	43	57		50	113	120	113	
715-9	69	62	64	83	62	64	64	66
715-11	139	125	136	122	115	120	146	
716-7	92	90	94	90	90	87	90	92
719c-5		113	113	113	115	115		154
719c-7	136	115	115	141	115	120	115	
720c-5		111		115	146	115	113	115
720c-6	64	129				62		71
720c-8	115	146	108	115	115	87	85	139
74-768	64	64	66	62	62	64	64	59
74-769	27	27	24	28	36	29	38	27
74-780	45	38	41	43	48	38	6	41
4384	134	148	127	125	129	148	125	

A-8. Life span in days after planting, polycross nursery, 1975.

Clones	Replications							
	1	2	3	4	5	6	7	8
74-770	188	161	161	164	169	157	154	172
74-771	168	202	178	172	164	188	185	172
74-772	136	188	161	154	157	136	157	154
74-773	188	113	178	185	157	136	129	161
74-774	169	188	188	198	157	169	188	178
74-776	169	172	188	178	172	185	164	157
74-777	169	190	178	161	188	172	169	161
74-778	188	136	157	188	129	169	172	157
74-779	188	188	169	185	169	159	178	148
74-781	164	169	178	154	157	169	161	154
74-782	178	161	157	188	161	157	172	169
74-783	178	172	161	172	178	161	172	161
74-785	154	188	185	134	154	158	172	161
74-786	178	161	161	169	161	148	154	157
74-787	188	188	185	169	188	157	161	169
74-788	178	188	161	185	178	161	171	154
74-789	198	198	178	198	169	188	161	164
74-790	169	198	185	169	188	157	161	161
74-791	188	178	169	185	188	157	169	129
74-793	178	188	188	188	161	157	169	178
462	202	202	202	199	188	199	199	202
516c-4	199	188	178	188	188	188	178	164
516c-7	188	198	199	198	198	178	178	161
74-559-1	199	172	188	199	188	198	185	198
570n	198	188	198	169	188	199	172	198
628-4	188	190	188	188	178	172	172	178
74-639-7	202	188	188	190	188	178	190	188
715-8	169	188	136	178	172	188	185	161
715-9	198	188	169	198	188	169	178	169
715-11	198	198	185	189	188	178	178	161
716-7	188	202	198	188	185	188	188	161
719c-5	188	188	188	185	188	190	185	190
719c-7	198	199	188	178	185	185	178	161
720c-5	129	202	161	185	185	188	178	188
720c-6	188	188	161	157	154	164	169	188
720c-8	199	169	188	198	188	178	188	190
74-768	188	188	175	175	188	175	188	161
74-769	172	188	188	157	188	169	178	161
74-780	188	198	188	185	185	154	178	161
4384	202	198	202	190	188	188	190	178

A-9. Total seed yield in grams per plant, harvested from the polycross nursery, 1975.

Clones	Replications 1	2	3	4	5	6	7	8
74-770	1.73	2.20	3.25	5.60	4.68	2.37	5.23	4.50
74-771	14.06	28.41	3.19	7.86	9.76	11.64	5.61	3.28
74-772	3.01	15.47	3.23	5.54	4.84	1.52	2.71	1.42
74-773	1.90	0.08	2.41	4.26	1.69	1.38	0.25	3.18
74-774	1.06	0.54	5.00	2.16		3.12	3.07	7.44
74-776	2.13	3.74	10.57	14.74	6.18	6.55	5.71	3.33
74-777	2.63	5.81	8.64	2.03	4.74	1.03	8.03	1.62
74-778	0.86	1.00	0.65	2.09	0.03	1.76	2.62	2.47
74-779	3.09	5.09	2.62	4.26	1.85	0.55	4.57	3.00
74-781	0.33	4.43	4.10	1.22	13.20	0.96	2.43	1.80
74-782	3.37	4.01	0.63	4.99	2.83	1.06	2.36	6.25
74-783	4.60	6.90	3.02	8.40	8.30	2.42	7.49	2.52
74-785	0.16	8.97	14.79	1.91	3.45	3.80	4.99	2.92
74-786	2.25	2.65	5.25	2.15	3.77	1.68	2.28	2.08
74-787	3.50	3.82	4.60	0.80	4.83	4.56	4.22	5.36
74-788	3.60	7.35	3.84	2.52	4.13	4.64	9.02	3.75
74-789	3.07	12.07	4.11	6.69	3.23	8.65	2.00	5.27
74-790	1.67	12.23	7.64	2.49	5.24	1.82	5.73	5.79
74-791	12.25	20.06	6.77	7.68	11.34	7.78	14.03	0.12
74-793	1.36	2.96	5.06	1.98	0.60	0.16	1.67	0.79
462	1.58	0.42	15.09	4.28	0.47	3.66	2.65	3.40
516c-4	10.21	27.40	29.86	16.60	10.65	29.96	11.43	26.31
516c-7	5.32	19.98	26.13	16.95	14.52	2.11	17.47	8.50
74-559-1	8.75	29.70	11.20	19.55	2.65	9.33	26.56	5.40
570n	3.89	1.94	17.20	0.40	9.66	2.36	0.00	0.60
628-4	7.80	6.69	16.86	9.83	11.05	5.27	3.48	2.92
74-639-7	16.28	10.08	11.84	23.76	23.60	11.18	21.66	14.44
715-8	2.90	4.89		7.30	2.60	0.54	4.92	
715-9	1.84	5.91	1.49	2.01	3.47	0.39	0.60	2.67
715-11	1.51	4.66	1.38	1.86	8.29	2.04	0.00	
716-7	14.24	41.72	15.32	18.32	18.27	17.40	18.78	2.83
719c-5		7.05	2.24	2.72	3.80	0.34		0.06
719c-7	0.09	1.93	11.88	0.00	5.43	0.21	0.48	
720c-5		3.78		0.12	0.00	1.29	2.26	2.54
720c-6	0.69	0.43				0.30		0.36
720c-8	6.81	0.00	9.84	3.97	6.86	4.05	5.97	3.00
74-768	0.22	1.36	0.33	1.51	0.66	0.75	1.91	0.20
74-769	4.94	5.60	7.76	0.60	3.23	3.25	10.38	5.78
74-780	6.60	10.67	6.33	6.06	8.96	3.80	10.74	5.66
4384	2.37	0.00	5.75	5.84	5.47	0.34	7.29	

A-10. Analysis of variance (mean squares) of the responses studied in the polycross nursery, 1975.

Source	df	Diameter of plants	Height of plants	Length of stol. inter.
Blocks	7	455.49**	10.8281**	0.3076
Genotypes	3			
Glucosides (G)	1	149.39	1138.0714**	3.7393**
Enzyme (E)	1	1896.41**	304.1575**	33.2051**
G x E	1	498.36*	89.6196**	3.7758**
Within genotypes	36			
Geno. liliacac	4	1314.73**	105.9150**	3.0275**
Geno. Li_ acac	4	383.60**	32.5875**	1.8725**
Geno. liliAc	6	1802.75**	107.8217**	3.8633**
Geno. Li_ Ac_	22	561.26**	23.5432**	2.5668**
Error	273	106.21	3.1003	0.3029

Source	df	Virus symp. rating	Flowering time +	Life span
Blocks	7	1.9674	738.36	1113.77**
Genotypes	3			
Glucosides (G)	1	6.1459*	38550.18**	5864.04**
Enzyme (E)	1	16.1021**	53597.98**	4385.20**
G x E	1	0.0010	6515.84**	883.87*
Within genotypes	36			
Geno. liliacac	4	1.9125	4802.55**	83.75
Geno. Li_ acac	4	9.5375**	8644.42**	764.00**
Geno. liliAc_	6	17.2800**	9356.27**	378.50**
Geno. Li_ Ac_	22	5.7609**	2007.31**	559.91**
Error	273	1.0388	607.86	159.35

Source	df	Seed yield	df	Flowering time ++
Blocks	7	102.7608**	7	48.64
Genotypes	3		3	
Glucosides (G)	1	652.2053**	1	31985.42**
Enzyme (E)	1	4.4606	1	86215.48**
G x E	1	7.1755	1	16605.07**
Within genotypes	36		36	
Geno. liliacac	4	450.2375**	4	6208.85**
Geno. Li_ acac	4	405.0025**	4	7855.48**
Geno. liliAc_	6	178.9183**	6	10712.58**
Geno. Li_ Ac_	22	96.2182**	22	2215.74**
Error	273	17.7650	259	199.92

* Significant at the 5% level
** Significant at the 1% level
+ Considering missing values as zeros
++ Omitting missing values.

A-11. HCN content in ppm, progeny test, 1976.

Progenies	Replications			
	1	2	3	4
74-770	2.50	2.50	3.00	2.25
74-771	1.75	4.00	3.00	2.25
74-772	2.75	5.50	2.75	2.50
74-773	4.00	3.25	4.00	4.75
74-774	4.75	3.50	2.50	1.75
74-776	3.25	3.25	4.00	2.50
74-777	1.75	3.00	4.00	3.50
74-778	1.25	1.50	2.00	1.75
74-779	2.50	6.00	2.75	2.75
74-781	1.75	2.25	2.00	1.50
74-782	7.50	4.00	2.75	3.50
74-783	4.75	2.25	4.25	1.50
74-785	3.00	2.75	2.00	3.00
74-786	2.75	2.25	2.25	2.25
74-787	5.50	4.50	2.75	2.75
74-788	4.25	1.75	2.00	1.75
74-789	1.50	2.00	3.00	1.25
74-790	2.50	2.00	2.00	2.00
74-791	2.75	2.25	3.50	3.50
74-793	1.75	1.75	2.75	3.25
462	0.75	0.50	0.75	1.50
516c-4	5.50	2.00	2.50	1.50
516c-7	0.50	1.00	1.00	1.50
74-559-1	1.50	1.00	1.50	1.25
570n	1.00	0.50	1.25	1.25
628-4	1.75	1.25	1.50	0.75
74-639-7	2.25	2.25	1.50	2.00
715-8	1.75	1.25	2.00	1.25
715-9	1.25	1.50	1.75	1.50
715-11	1.75	1.25	1.75	1.25
716-7	1.00	1.00	1.50	1.25
719c-5	1.00	1.50	1.25	0.75
719c-7	0.50	0.75	1.75	1.50
720c-5	1.25	0.50	1.25	1.50
720c-8	3.75	1.00	1.00	1.25
74-769	1.25	1.75	0.50	2.50
74-780	1.75	2.00	1.50	1.25
4384	1.00	1.25	1.25	1.25

A-12. Flowering time in days after planting, progeny test, 1976.

	Replications			
Progenies	1	2	3	4
74-770	133	138	130	130
74-771	126	147	132	130
74-772	138	117	122	130
74-773	131	135	128	128
74-774	136	135	135	138
74-776	151	142	135	128
74-777	134	132	132	134
74-778	135	135	138	128
74-779	138	132	126	130
74-781	128	144	132	132
74-782	122	135	117	142
74-783	122	122	123	135
74-785	135	132	128	122
74-786	135	144	155	130
74-787	132	128	140	130
74-788	122	135	147	122
74-789	126	131	138	128
74-790	138	128	122	134
74-791	138	138	128	128
74-793	138	138	138	123
462	155	140	145	138
516c-4	148	135	122	134
516c-7	198	198	192	197
74-559-1	145	143	138	138
570n	144	144	148	140
628-4	130	148	138	138
74-639-7	135	135	138	138
715-8	138	148	135	143
715-9	127	140	132	128
715-11	142	142	145	138
716-7	124	140	138	134
719c-5	142	142	145	138
719c-7	132	155	144	148
720c-5	138	148	151	135
720c-8	142	132	132	128
74-769	140	132	122	122
74-780	126	144	123	119
4384	130	140	148	140

A-13. Length of stolon internodes in cm, progeny test, 1976.

Progenies	Replications			
	1	2	3	4
74-770	1.2	4.5	3.5	4.5
74-771	4.0	3.0	3.2	4.0
74-772	3.0	2.5	3.7	5.2
74-773	1.5	3.0	3.0	6.0
74-774	4.5	4.5	4.0	2.2
74-776	2.0	2.0	2.8	4.2
74-777	2.5	2.5	4.5	4.2
74-778	3.0	3.0	2.8	4.0
74-779	3.5	4.3	4.0	4.0
74-781	1.5	2.2	3.2	5.0
74-782	5.5	2.2	1.8	1.7
74-783	4.5	3.2	5.0	2.2
74-785	3.5	4.5	4.0	4.0
74-786	2.3	3.0	4.2	6.0
74-787	3.5	3.4	4.8	4.0
74-788	2.5	4.5	2.5	3.0
74-789	3.0	4.0	3.2	5.5
74-790	2.0	6.0	5.0	4.4
74-791	4.0	3.0	2.8	5.0
74-793	4.0	3.0	3.5	5.2
462	2.0	5.0	3.0	2.0
516c-4	3.0	4.7	6.0	3.0
516c-7	5.5	2.5	2.0	3.0
74-559-1	4.0	2.2	3.0	2.3
570n	2.0	2.0	3.0	4.0
628-4	1.7	1.8	2.2	2.5
74-639-7	2.0	2.5	2.6	4.2
715-8	2.8	3.0	3.5	3.5
715-9	2.0	2.2	3.2	2.7
715-11	2.5	2.5	2.8	3.8
716-7	3.5	2.8	2.7	3.0
719c-5	2.8	3.8	2.5	2.5
719c-7	1.8	1.8	2.5	5.7
720c-5	2.5	3.0	4.5	3.5
720c-8	2.0	5.5	3.5	4.7
74-769	4.0	3.0	4.5	3.4
74-780	3.5	4.0	3.8	3.5
4384	1.5	2.5	1.4	3.0

A-14. Dry matter yield in kg/ha, first harvest, April 13, 1976.

Progenies	Replications							
	1		2		3		4	
	Grass	Clover	Grass	Clover	Grass	Clover	Grass	Clover
74-772	786.3	0.0	388.9	1111.1	443.6	118.3	382.4	48.7
74-773	324.2	0.0	341.2	22.3	731.1	855.6	659.8	370.7
74-774	740.0	1840.0	639.6	823.6	663.1	37.9	444.8	37.1
74-777	362.5	7.4	325.9	111.1	519.3	155.8	333.3	151.5
74-779	742.1	16.0	622.6	207.5	133.5	29.7	658.9	96.2
74-782	471.8	3448.1	342.1	7.4	237.9	65.6	512.5	8.0
74-783	455.6	511.1	229.4	22.2	370.1	222.1	667.7	29.7
74-787	592.2	2791.9	369.3	66.5	458.8	15.6	476.8	523.0
516c-4	450.9	14.8	415.2	103.9	685.2	1388.9	727.9	388.2
720c-8	768.3	687.4	518.5	59.3	673.7	118.4	600.0	22.2
720c-5	398.0	9.3	614.4	37.0	516.6	137.2	464.7	167.6
74-780	817.8	1848.9	525.9	29.6	549.0	111.3	296.1	88.8
74-559-1	556.4	59.3	466.1	44.4	570.4	611.1	256.6	108.9
715-9	599.6	37.0	714.8	0.0	674.7	364.5	222.2	266.7
719c-5	486.6	9.5	533.3	259.3	710.4	320.5	922.9	209.4

A-15. Dry matter yield in kg/ha, second harvest, May 11, 1976.

Progenies	Replications							
	1		2		3		4	
	Grass	Clover	Grass	Clover	Grass	Clover	Grass	Clover
74-772	363.0	7.4	260.7	308.1	231.1	62.2	312.3	72.1
74-773	237.0	0.0	192.6	7.4	391.1	497.8	284.4	213.3
74-774	422.2	1041.5	405.4	354.8	222.2	22.2	214.0	24.7
74-777	353.6	9.6	266.7	118.5	316.2	162.4	214.8	140.7
74-779	508.1	93.3	296.3	174.3	203.7	64.8	245.2	30.6
74-781	364.7	38.0	279.8	0.0	244.4	51.8	313.2	42.3
74-782	131.0	1114.0	171.5	7.8	304.5	74.1	192.6	7.4
74-783	302.1	302.1	192.6	59.3	328.8	160.0	348.2	66.7
74-787	358.5	1010.4	303.7	51.8	264.9	17.1	313.7	418.3
74-639-7	303.7	22.2	241.5	38.6	192.6	140.7	296.3	405.5
516c-4	251.8	22.2	327.3	103.4	325.3	650.7	339.9	287.6
628-4	251.9	148.2	359.1	35.9	452.7	49.4	391.1	248.9
720c-8	560.0	666.7	311.9	78.0	274.1	103.7	140.7	14.8
516c-7	435.7	813.4	244.4	14.8	318.5	22.2	296.3	125.9
720c-5	240.2	41.4	410.3	59.8	239.3	239.3	322.8	205.4
74-780	641.0	997.1	321.0	24.7	362.9	74.1	244.4	177.8
74-559-1	246.9	138.9	348.1	88.9	311.1	400.0	207.4	290.4
715-9	425.2	114.1	200.0	51.9	386.7	266.7	220.5	194.0
716-7	296.3	260.7	250.0	46.3	229.6	103.7	260.8	320.0
719c-5	185.2	37.0	346.7	635.5	251.8	414.8	455.8	356.1

A-16. Dry matter yield in kg/ha, third harvest, June 8, 1976.

Progenies	Replications							
	1		2		3		4	
	Grass	Clover	Grass	Clover	Grass	Clover	Grass	Clover
74-772	568.9	26.7	577.8	251.8	346.7	133.3	273.6	45.6
74-773	568.9	17.8	447.4	23.1	563.0	266.7	466.7	213.3
74-774	311.1	370.4	539.3	394.0	426.7	97.8	616.3	142.2
74-777	437.0	14.8	577.8	192.6	573.3	186.7	435.6	165.9
74-779	628.2	71.1	520.0	213.3	415.6	37.1	518.5	62.2
74-781	393.4	38.6	368.3	0.0	236.8	39.5	508.2	83.0
74-782	404.4	539.3	415.5	7.4	408.2	59.4	344.3	8.0
74-783	444.4	311.1	377.6	74.0	628.2	59.3	509.6	142.2
74-787	391.1	391.1	384.7	14.8	429.6	29.6	493.3	253.3
74-639-7	385.2	37.0	425.2	134.8	444.4	281.5	481.5	520.0
516c-4	308.6	86.9	509.6	142.2	560.0	497.8	414.8	225.2
628-4	363.0	197.0	379.2	33.0	488.9	62.2	603.0	277.0
720c-8	554.0	554.0	509.6	142.2	404.4	134.8	296.3	14.8
516c-7	352.6	539.3	339.8	14.8	444.4	29.6	693.3	213.3
720c-5	437.0	37.0	487.4	92.9	429.6	325.9	337.9	426.7
74-780	358.5	505.2	515.6	44.4	663.7	59.3	462.2	201.5
74-559-1	426.7	213.3	422.2	59.3	643.0	373.3	343.7	248.9
715-9	364.4	97.8	429.6	37.0	474.0	281.5	355.6	340.7
716-7	231.1	204.4	586.7	586.7	422.2	533.3	521.5	309.6
719c-5	440.0	325.9	363.5	51.9	391.1	260.7	379.3	260.7

A-17. Analysis of variance (mean squares) of the responses studied in the progeny test.

Source	df	Length of stol. int.	Flowering time	HCN
Blocks	3	5.3434**	613.63	1.7614
Progeny	3			
Glucosides (G)	1	1.8041	13.43	12.5894**
Enzyme (E)	1	6.9805*	3469.75**	38.3939**
G x E	1	0.0268	1823.28*	7.5729**
Within progeny	34			
Geno. liliacac	4	0.1333	112.88	0.1453
Geno. Li_ acac	4	2.7258	81.30	2.2297*
Geno. liliAc_	5	1.1764	2268.94**	0.2401
Geno. Li_ Ac_	21	0.7812	53.82	2.5173**
Error	111	1.1086	452.30	0.7845

		Dry matter yield, April 13, 1976.		
Source	df	Grass	Clover	Total
Blocks	3	26251	1106858	1430249
Progeny	3			
Glucosides (G)	1	82698	101353	944
Enzyme (E)	1	160	217341	205656
G x E	1	20367	2631	37662
Within progeny	11			
Geno. liliacac	2	40504	1743	40691
Geno. Li_ acac	1	9891	127134	66102
Geno. liliAc_	1	4758	373032	462049
Geno. Li_ Ac_	7	25445	427419	463179
Error	42	29629	442605	534521

A-18. Analysis of variance (mean squares) of the second, and third harvests, and total dry matter yield.

Source	df	Dry matter yield, May 11, 1976.		
		Grass	Clover	Total
Blocks	3	16896	193108*	316364*
Progeny	3			
Glucosides (G)	1	495	1118	3097
Enzyme (E)	1	2773	26436	46320
G x E	1	38014*	946	40941
Within progeny	16			
Geno. liliacac	3	2404	32953	42676
Geno. Li_ acac	2	3099	21810	8664
Geno. liliAc_	2	8721	33474	73582
Geno. Li_ Ac_	9	4791	55985	56547
Error	57	8408	69595	96784

Source	df	Dry matter yield, June 8, 1976.		
Blocks	3	9802	40199	25928
Progeny	3			
Glucosides (G)	1	6209	42223	16049
Enzyme (E)	1	1238	59139	43266
G x E	1	3002	161	1772
Within progeny	16			
Geno. liliacac	3	3658	39473	52507
Geno. Li_ acac	2	308	9767	7409
Geno. Li_ Ac_	9	10267	16070	28839
Error	57	10980	26825	45114

Source	df	Total dry matter yield, 1976.		
Blocks	3	35242	2194436	2735116
Progeny	3			
Glucosides (G)	1	11130	14380	208
Enzyme (E)	1	1611	5067	12391
G x E	1	20650	7430	3306
Within progeny	16			
Geno. liliacac	3	353097**	53299	511460
Geno. Li_ acac	2	399694**	512390	1662001
Geno. liliAc_	2	450696**	476527	1622468
Geno. Li_ Ac_	9	271886**	933891	1542728
Error	57	67173	892696	1103243

* Significant at the 5% level
** Significant at the 1% level.

BIOGRAPHICAL SKETCH

Nilton Rodrigues Paim was born on April 8, 1946, in Vacaria, Rio Grande do Sul, Brazil. He graduated from high school in 1965. From March 1966 to December 1969 he attended the Universidade Federal do Rio Grande do Sul, receiving the degree of Engenheiro Agronomo. In 1970 he started the graduate school at the same University and received the degree of Master of Science with a major in Agronomy in November of 1971. In December of 1971 he joined the staff of the Departamento de Fitotecnia of the Universidade Federal do Rio Grande do Sul to work with forage crops and plant breeding. In 1974 he received a scholarship from USAID/University of Wisconsin - Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul Contract to continue his studies toward the degree of Doctor of Philosophy with a major in Agronomy.

He is married to the former Maria Isabel Silva de Athayde Bohrer and they have a son, Felipe. The author is a member of the Sociedade Brasileira de Zootecnia, Sociedade Brasileira de Genetica and American Society of Agronomy.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

C. E. Dean

C. E. Dean, Chairman
Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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